The effect of feeding varying inclusion levels of velvet bean (*Mucuna pruriens*) seed meal on growth performance and physicochemical attributes of broiler chicken

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DECLARATION

I, Makiwa Simeon Mthana, Student Number: 201206944, hereby declare that this dissertation has never been previously submitted to any University and this is my original work conducted under the supervision of Mr C.S. Gajana and co-supervision of Dr. B. Moyo and Prof. J.F. Mupangwa. All references contained have been acknowledged.

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ABSTRACT

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This study was conducted to determine the effect of feeding varying inclusion levels of velvet bean (*Mucuna pruriens*) seed meal (VBSM) on growth performance and physicochemical attributes of broiler chicken. *Mucuna pruriens* seed is an indigenous legume seed commonly found in tropical and sub-tropical areas; however, it can also be planted and cultivated. It is a viable source of dietary protein with an average concentration of 33.4%. Soybean is expensive. In addition, the South African production levels do not meet the current demand; hence the majority of soybean oilcake is imported. Recently, consumers have been conscious of the quality of chicken meat from both local and international producers. Therefore, there is a need to explore velvet bean that can grow in poorly fertilised and low rainfall areas. The study was conducted at Fort Cox Agriculture and Forestry Training Institute. One hundred and twelve (112) Cobb broilers were used, with an average weight of 45.2g. Four diets were formulated to be iso-caloric and iso-nitrogenous comprising velvet beans at 0, 10, 15 and 20 % (T1, T2, T3 and T4). The broilers were reared for 42 days with 35 days on experimental diets. Feed intake, body weight gain, feed conversion ratio, mortality, final body weight, carcass weight, and dressing percentage were determined. At day 42, twenty birds per treatment were randomly selected and fasted for five hours with water offered *ad libitum*. After slaughter the meat pH_{24}, colour (L*, a*, b*), Warner-Bratzler shear force (WBSF), thawing loss (TL), and cooking loss (CL) measurements were performed on 80 breast and 80 thigh muscles. The data for growth performance and physicochemical parameters was analysed using General Linear Model (GLM) procedure of SAS (SAS, 2006), mean separation was done using LSD test option of SAS. Daily feed intake, body weight gain, final weight and carcass weight of broilers from T1 were significantly higher ($P < 0.05$) from other treatments. Daily feed intake (DFI), and body weight gain (BWG) in T1 were also higher ($P < 0.05$) in both phase 1 and phase 2 compared to other treatments. Feed conversion ratio (FCR) was not influenced by diets ($P > 0.05$). High ($P < 0.05$) mortality was observed on broilers fed diet with 0% VBSM compared to those fed diets comprising of VBSM. The diets did not have an effect ($P > 0.05$) on thigh meat pH_{24}, lightness, redness, and WBSF. Cooking loss (25.69%) was higher ($P < 0.05$) in thigh meat of broilers fed the control diet. Breasts shear force (14.20) was higher in T4. Cooking loss of breast meat from broilers on T1 and T2 was higher ($P < 0.05$) than those from other treatments. Breast meat colour ($P > 0.05$) was not influenced by diets. It can be concluded that the VBSM has an effect on feed intake, growth performance and mortality of broilers, except on FCR and dressing percentage. It was also concluded that the VBSM can be included in broiler diets up to the level of 15% without negatively affecting the quality of meat.

Keywords: Body weight gain, feed intake, Meat colour, *Mucuna pruriens*.

DEDICATION

I dedicate this work to my late father (Gideon Mucheki) and brother (Thabo Ranero Mucheki), may their souls rest in peace.

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LIST OF ABREVIATIONS

a*	Redness of meat
ADF	Acid detergent fibre
ADFI	Average daily feed intake
ADG	Average daily gain
ADL	Acid detergent lignin
ADWG	Average daily weight gain
ANF	Anti-nutritional factors
ANOVA	Analysis of variance
AOAC	Association of Official Analytical Chemists
b*	Yellowness of meat
CF	Crude fibre
CL	Cooking loss
CP	Crude protein
DAFF	Department of Agriculture Forestry and Fisheries
DFD	Dark firm and dry
DM	Dry matter
EE	Ether extract
FCR	Feed conversion ratio
FI	Feed intake

GLM	Generalized Linear Model
L*	Lightness of meat
L-Dopa	Levodopa
LSD	Least Significance Difference
ME	Metabolisable energy
NDA	National Department of Agriculture
NDF	Neutral detergent fibre
NFE	Nitrogen-free extract
PEG	Polyethylene glycol
pH_{24}	Meat pH after 24 hours of slaughter
PSE	Pale, soft, and exudative
SAPA	South African Poultry Association
SAS	Statistical analysis system
TL	Thawing loss
VBS	Velvet bean seed
VBSM	Velvet bean seed meal
WBSF	Warner bratzler shear force

CHAPTER ONE: Introduction

1.1. Background

Broiler production is one of the largest sectors by 16.2 to 17% from 2012 to 2017, contributing about 1.7% of the total worth of agricultural goods in South Africa (DAFF, 2013; NDA, 2018). This clearly shows that recently in South Africa, poultry meat production as a protein source for human consumption is increasing. Chicken meat was the cheapest animal protein source, with an average price of R18.92/kg (SAPA, 2016). Due to this affordability of chicken meat when comparing with other meat types (beef, mutton, and pork); the consumer demand has increased over the years. In the world perspective, van de Poel *et al.* (2013) state that chicken meat is the leading meat that is consumed in the world. Gunya (2016) also indicates that the alleged health related issues attached to red meat by consumers have increased the demand for chicken meat. Therefore, there is a dire need to improve chicken production and quality in order to meet the huge demand.

Velvet bean seed (VBS) is one of the indigenous legumes commonly found in both hot and cold habitats of the world, but it can also be planted and be cultivated. The seeds are considered as a sustainable source of protein because of their high crude protein content that ranges between 25.7 and 43.5% (Balogun and Olatidoye, 2012; Tavares *et al*., 2016). In addition, according to Gurumoorthi *et al*. (2003), when comparing VBS with other legume seeds such as lima bean, rice bean, and soybean; its digestibility is higher. Pods and seeds can be milled and fed to both ruminants and mono-gastric animals, while leaves and stems can be used as pastures, hay, or silage for ruminants (Ani, 2008). In some countries, the seeds are used as a cover-crop item where they are grown to protect and enrich the soils. Individuals traditionally consuming velvet bean claim that it has various medical properties (Jayaweera *et al*., 2007), but that effect has not yet been demonstrated in animals.

Feed is one of the most costly inputs in the poultry production. Tiroesele and Moreki (2012) states that in poor managed broiler farms the cost of feed for broilers can amount to 70% of their total production costs, which will eventually result in lower turnover. Currently, the protein sources in broiler feeds such as fishmeal, sunflower meal and soybean meal are expensive (Prayogi, 2011). The supply of affordable feed from feed manufacturing industries is very low, due to the escalating costs of feed ingredients (Tiroesele and Moreki, 2012). Furthermore, the conventional protein supplements are not only consumed by poultry but also by human beings, thus promoting a competition between poultry and human beings. The major challenge in poultry production is the availability of good quality feed at cheaper prices. Commercial poultry feed manufacturers depend on scarce and expensive conventional feed ingredients such as fishmeal, soybean meal and sunflower meal. Thus, this has resulted in the increase of the production cost of broilers (Tiroesele and Moreki, 2012).

1.2. Problem Statement

Both commercial and smallholder broiler enterprises are growing, which results in an increase in the demand for broiler feed. Soybean meal and sunflower meal have customarily been the leading protein sources in broiler diets, but the increasing demand has failed to sustain broiler feed industries, hence they end up relying on imported protein sources. Soybean meal among other common legume seeds has been the leading protein source in broiler diets in South Africa, but its production in South Africa is not satisfactory to meet the protein source demands of the expanding poultry industries. The growing demand by health conscious consumers for broiler meat of good quality (Al-Hassan *et al.*, 2014) has caused pressure on nutritionists to explore other potential alternatives such as VBS that can be used to replace the currently available expensive conventional protein sources.

1.3. Justification

From literature, crude protein content of VBS can vary from 25.7 to 43.1% (Balogun and Olatidoye 2012; Tavares *et al*., 2016), hence it may be a possible source proteins. Furthermore, feeding VBSM on broilers has been widely investigated by scientists (Del Carmen *et al*., 1999; Iyayi and Taiwo, 2003; Nyirenda *et al*., 2003; Jayaweera *et al*., 2007; Ani, 2008; Vadivel and Pugalenthi, 2010; Mangwiro *et al*., 2013). However, most of these studies were carried out in East Africa and Asia. No studies of this nature have been conducted in Southern Africa to our knowledge. Currently, there is inadequate data on the influence of feeding VBSM on the quality of meat; hence such evidence is important in Southern Africa.

The *Mucuna pruriens* seed contains anti-nutritional factors (ANF) that may hinder growth of birds. Scientists have shown several processing techniques that can be used to diminish those bioactive compounds (Vadivel and Pugalenthi, 2010; Mugendi *et al*., 2010). However, these bioactive compounds can act as potent antioxidants that contribute largely to good quality meat (Seeram *et al*., 2005; Reddy *et al*., 2007). The consumption of chicken meat from broilers fed diets with VBSM is expected to have a good effect on reducing protein malnutrition as well as decreasing health hazards. Furthermore, various researches regarding VBS have been carried out showing a cholesterol lowering effect (Iauk *et al*., 1989; Del Carmern *et al*., 1999; Jayaweera *et al*., 2007). However, this claim has not been fully investigated. Therefore, there is a paucity of information on the influence of this particular nutritious ingredient on broiler growth presentation and meat quality in Southern Africa.

1.4. Objectives

The broad objective of the study was to determine the effect of diets with varying inclusions of velvet bean (*Mucuna pruriens*) seed meal on broiler growth performance and physicochemical attributes of meat.

Specific objectives

The specific objectives of the study were:

1. To determine the effect of feeding varying inclusion levels of heat processed velvet bean (*Mucuna pruriens*) seed meal on feed intake and growth performance of broilers.
2. To determine the effect of feeding varying inclusion levels of heat processed velvet bean (*Mucuna pruriens*) seed meal on physicochemical attributes of broiler meat.

1.5. Null hypothesis

1. Feeding varying inclusion levels of heat processed velvet bean (*Mucuna pruriens*) seed meal does not influence feed intake and growth performance of broilers.
2. Feeding varying inclusion levels of heat processed velvet bean (*Mucuna pruriens*) seed meal does not alter physicochemical attributes of broiler meat.

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CHAPTER TWO: LITERATURE REVIEW

2.1. Introduction

Legume seeds or pulses have become an important cheap protein source in developing countries (Kamatchi *et al*., 2010). They are not only rich in protein, but they also provide sufficient concentration of minerals, carbohydrates and vitamins (Siddhuraju *et al*., 2002). Because of high nutrients, various legume seeds are included in animal feed formulations. Velvet bean (*Mucuna pruriens*) seeds have been used in both human and animal diets as they have the ability to supply some protein (Del Carmen *et al*., 1999; Taylor 2003), carbohydrates, lipids, fibre and minerals (Taylor, 2003). Currently, there is high competition for food resources, especially conventional protein sources between human and broiler industries. This has led to inadequate supply of protein sources in the local market, or if present, protein sources are usually expensive (Pugalenthi *et al*., 2005). Ukachukwu and Sbza (2003) noted that soybean and fish meal are major source protein in broiler diets in the world. Therefore, their scarcity results in increased price of broiler feed and eventually affect the take-off of the broiler industries (Ukachukwu, 2007). There has been a surge in research focus on finding affordable alternative protein sources to meet the demand for protein ingredients by the broiler industry. Voisin *et al*. (2014) conducted some research investigations on the possibilities of using underutilised legume seeds such as velvet bean as an additional dietary protein in broiler diets.

In most parts of the world, velvet bean farmers use the plant as a cover crop and as a soil improver. Del Carmen *et al*. (1999) and Ani (2008), state that the bean is rich in protein but contains a toxic amino acid called L-Dopa and other bioactive compounds that depress digestibility of nutrients by broilers. In order to improve digestibility, the toxic substances can be destroyed by heating (boiling, roasting and grilling) and other processing techniques such as fermentation, acid and base soaking, and germination (Mugendi and Njagi, 2010).

The seed coat colour is directly related to high concentration of polyphenols. Gurumoorthi *et al*. (2003) supports this fact as they observed that black coated VBS accessions had higher tannin and phenolic content compared to white coated seeds. Velvet bean seed is not a conventional protein source in commercial broiler diets because of these inherent anti-nutritional factors that can be eliminated and because of consumers' lack of knowledge concerning it. Feeding VBSM to broilers has been widely investigated (Del Carmen *et al*., 1999; Iyayi and Taiwo, 2003; Nyirenda *et al*., 2003; Jayaweera *et al*., 2007; Ani, 2008; Vadivel and Pugalenthi, 2010; Mangwiro *et al*., 2013). However, the focus has been on growth performance and little has been done on other parameters such as meat quality. Therefore, further research on indigenous legume seeds would provide more knowledge, which will help to alleviate critical food shortage, expand the food base, and improve income generation especially for smallholder farmers. The review seeks to summarise the current research on the influence of VBSM as a source protein in poultry feed, and its challenges and benefits on broiler growth performance and meat quality.

2.2. Velvet bean plant and seed description

Velvet bean seed is a legume seed fitting to the *Fabaceae* family. Its scientific name is *Mucuna pruriens* and its common names include cow-itch, cowhage, cowage, itchy bean, and krame (Lampariello *et al*., 2012; Tropical plant database, 2017). The plant is an annual legume that grows to a height of about 3-18 meters. In most tropical regions, especially in India and Africa, the plant is indigenous (Tropical plant database, 2017). The bean grows well in a warm temperature of about 20-30°C throughout the growing period. The velvet bean is vulnerable to frost and it usually requires a 180 to 240 days frost-free period with annual rainfall of 380 mm (Bachmann, 2017). It also grows well on sandy soils as well as on clay soils with a pH of 5.0 to 6.5 (Wulijarni and Maligalig, 1997). The bean seeds are enclosed in shells, and the resulting pods are covered with reddish-orange hair like structures that can cause extreme irritation to the skin (Maldonado, 2016). Each pod contain 4 to 6 seeds, which are globular in shape and approximately 1.2 x 1.2 cm in size, with the weight of 0.55 to 0.85 g/seed; in most cases they have a black colour or mottled brown, and sometimes with a greyish background (Lampariello *et al.*, 2012; Bachmann, 2017). The distinctive white aril surrounds the funicular hilum which is about 4 mm long and lastly the seed coat is thick, hard and glossy (Maldonado, 2016).

2.3. Nutritional composition of velvet bean

Bean seeds play a major part in the nutrition of both animals and human beings throughout the world. Not only is the endosperm nutritive, but the seed coat also. Ezeagu *et al*. (2003) concluded that the small seed coat might be the one causing chemical differences between *Mucuna pruriens* varieties. Velvet bean seeds possess good nutritional parameters and are excellent sources of micronutrients, protein, dietary fibre, bioactive compounds such as L-Dopa, tannins, total free phenolic, and phytic acid, oxalate, and low levels of fat (Vadivel *et al*., 2011; Vadivel and Biesalski, 2012). Table 2.1 and Table 2.2 below illustrate the proximate analysis and mineral composition of processed VBS as reported by Iyayi and Taiwo (2003); Ravindran, and Ravindran (1988); Balogun and Olatidoye (2012); Tavares *et al*., (2016), respectively.

Table 2.1: The proximate analysis of treated and untreated velvet bean seeds

Reference	Ravindran and Ravindran, (1988)	Iyayi amd Taiwo, (2003)	Balogun and Olatidoye, (2012)	Tavares *et al*. (2016)
Seed processing method	Raw	Autoclave at 120°C for 18 min	Raw	Oven heated at 50°C for 100 h
Proximate composition				
Crude protein (g/kgDM)	264	354	257	431
Crude fibre (g/kgDM)	63	77	72	56
Ether extract (g/kgDM)	41	32	14.5	70
Ash (g/kgDM)	37	36	36	31
Nitrogen-free extract (g/kgDM)	596	479	430	371
Metabolisable Energy (kcal/kg)	3375.18	3201.21	2544.61	3398.15

g/kg DM = grams/ kilograms Dry Matter, h= hours.

2.3.1. Protein

Protein is one of the important nutrient components in food. According to Hou *et al.* (2016), dietary protein supplies nitrogen and amino acids that are necessary for the functioning of organisms. Functional properties of an organism, such as oil and water absorption, foaming, nitrogen solubility, and emulsification are triggered by protein availability in animal feed (Maity *et al*., 2009). Due to the presence of bioactive peptides, protein is believed to have other specific functions, thus making it a good health-promoting nutrient for broilers (Grolichova *et al*., 2004). The crude protein (CP) concentration of raw VBS can be as low as 210 g/kg DM (Ferriera *et al*., 2003) and as high as 380 g/kg DM (Adebowale *et al*., 2005). These differences are due to factors such as variety, growth environment, and maturity stage. Nitrogen availability during bean filling and maturity affect final protein concentration in bean seeds. Yi and Lindner (2008) found that mature bean seeds had 240 g/kg DM CP content while immature ones had 370 g/kg DM. Bressani (2002) states that protein from VBS is highly made up of albumin and globulins, which are typically known to have a good profile of essential amino acids. The *Mucuna pruriens* crude protein of 256.5 g/kg DM is equivalent to other protein rich foods such as cowpeas, soybeans, pigeon peas, gourd seeds, and pumpkin that range from 231 to 330 g/kg DM (Olaofe *et al*., 1994).

Compared to cereal grains which are low in protein and lysine, VBS are good sources of supplementary protein for monogastric diets due to their high lysine concentration (Bressani, 2002; Adebowale *et al*., 2007). Other seeds such as sunflower, rice bean and lima bean have low digestibility compared to VBS (Friedman, 1996). Vadivel and Janardhanan (2000) observed that the *in vitro* protein digestibility of the seeds ranged from 72.4 to 76.9%. On the other hand, Mugendi and Njagi (2010) observed high levels of *in vitro* protein digestibility (80.5%) of processed VBS compared to raw seed, which was 67.2%. The physiological consumption of specific amino acid when ingested and absorbed determines the quality of protein (Oshidi *et al*., 1993; Friedman, 1996). The level of some of the essential amino acids such as valine, tyrosine, threonine, histidine, phenylalanine and isoleucine were established to be greater in most of the explored VBS (Siddhuraju *et al*., 1996; Siddhuraju and Becker, 2005; Gurumoorthi *et al*., 2008). In other studies, Oshodi *et al*. (1998); Aremu *et al*. (2006); and Balogun and Olatidoye (2012), reported that the major abundant amino acids were arginine, glutamic acid, and aspartic acid with the values of 7.41, 13.28, and 14.28 g/100 g proteins, respectively.

2.3.2. Lipids

Lipids are vital in animal nutrition since they promote fat-soluble vitamin absorption (Bogert *et al*., 1994). Higher amounts of lipids make processed VBS to be energy dense, thus they do not add to the bulk of the diet. The lipid concentration of VBS varies from reports of low ranges of 28 to 49 g/kg DM to higher ranges of 85 to 140 g/kg DM (Siddhuraju *et al*., 2002; Vijayakumari, *et al*., 2002). The crude lipid content of all the accessions of *Mucuna pruriens* seeds appears to be greater compared to other previously studied common legume seeds (Fathima *et al*., 2010). Adebowale *et al*. (2005) indicates that the lipid of seed coat, whole seed and cotyledon consists of 30, 96 and 98 g/kg DM lipids respectively. Soybean seed has about 235 g/kg DM of lipids, which is higher than that of velvet bean seeds 145 g/kg DM lipids (Balogun and Olatidoye, 2012).

2.3.3. Carbohydrates

There are other nutrients in velvet bean seeds, although they are found in small quantities with the exception of Nitrogen free extract (NFE) which is in large quantity (Iyayi and Taiwo, 2003) (Table 2.1). According to Balogun and Olatidoye (2012), velvet bean is a rich source of energy capable of supplying the daily energy requirements of animals because of its nitrogen free extract value of 429.8 g/kg DM. On the other hand, Ravindran and Ravindran (1988) indicated that raw and processed VBS contain nitrogen free extract and carbohydrate values of 596 and 429.8 g/kg DM respectively. According to Adepo *et al*. (2016), neutral detergent fibre (NDF), acid detergent fibre (ADF), and acid detergent lignin (ADL) of roasted VBS was found to be 32.73, 13.77, and 12.12 g/100gDM respectively, whereas, according to Feedipedia (2018), NDF, ADF, and ADL are 19.8, 7.5 and 0.9 g/100g, respectively. Bressani (2002) stated that NDF and ADF concentrations of VBS are at least as great as those of other legume seeds. Therefore, because of high levels of NDF VBS intake would slowly increase blood glucose levels and decrease plasma cholesterol due to fibre intake from the beans.

2.3.4. Minerals

Animal feed should contain adequate minerals, which are essential constituents of skeletal structures such as bones and cartilage development. According to the observation of Iyayi and Taiwo (2003) together with Balogun and Olatidoye (2012), VBS are composed of minerals ranging from 0.2 to 129 mg/kg DM depending on the type of mineral. The seeds are rich in some minerals as compared to other nuts and oil seeds and they have the following levels in mg/100g: Ca (148.88 – 710.42), Na (54.46 – 88.20), K (1472.33 - 1638.40), Mg (23.66 – 512.42), P (377.12 – 530.82), Fe (3.44 – 8.16), Zn (1.98 - 3.46), Cu (0. 54 – 0.72) (Fathima *et al*., 2010). Therefore, in terms of minerals, velvet bean is richer in calcium and average in copper. The high variations in nutrient composition (Table 2.2) may be due to maturity of the seeds, soil properties where the plant was planted, phenotypic variation of the seed, and the different seed processing methods.

Table 2.2: Mineral composition of treated and untreated velvet bean seeds

Reference	Ravindran and Ravindran, (1988)	Iyayi and Taiwo, (2003)	Balogun and Olatidoye, (2012)	Tavares *et al*. (2016)
Seed processing method	Raw	Autoclave at 120°C for 18 min	Raw	Oven heated at 50°C for 100 h
Macro minerals (g/kg DM)				
Potassium (K)	11.1	14	14.72	63.5
Calcium (Ca)	2.5	10	1.48	7
Magnesium (Mg)	1.1	19	0.23	0
Phosphorus (P)	2.2	8	3.8	8.3
Micro minerals (mg/kg DM)				
Zinc (Zn)	10	13	34.6	6
Manganese (Mn)	10	27	52.8	-
Iron (Fe)	13	129	34.4	153
Copper (Cu)	6	25	7.1	3

g/kg DM = grams/ kilograms Dry Matter, *mg/kg DM* = milligrams/kilogram Dry Matter.

2.4. Anti-Nutritional factors (ANFs) of velvet bean seeds

2.4.1 L-Dopa

Velvet bean seeds contain the most potential toxic anti-nutrient, a non-protein amino acid and pharmacologically active factor known as L-Dopa (Ani, 2008; Vadivel and Pugalenthi, 2010). Because of the presence of L-Dopa, the seed has been reported to cause severe dyskinesias and gastrointestinal disturbances such as vomiting in humans. When large amounts of improperly treated or processed VBS are ingested by broilers, they are identified to cause skin eruption and high body temperature (Flores *et al.*, 2002). Furthermore, animals with glucose-6-phosphate dehydrogenase in their red blood cells, levodopa is toxic, resulting in the stimulation of favism (Nechama and Edward, 1967). At high temperature and/or alkaline pH in a moist environment, L-Dopa is readily denatured (Gurumoorthi *et al.*, 2008). It is reported that available L-dopa in VBS may range from 0.43 to 7.48 as shown in Table 2.3 below. The utmost influences of VBS in monogastric animals are caused by L-Dopa absorption in the blood stream. Symptoms in broilers include lower feed intake, decreased body gain, and poor feed efficiency, when VBS are fed raw (Del Carmen *et al.*, 2002; Flores *et al.*, 2002). Flores *et al.* (2002) reported poor palatability, daily weight gains, feed intake, and feed conversion ratio when VBS were substituted for soybean meal in pig diets. Necropsy or post mortem revealed acute toxic hepatitis and advanced necrosis in pigs fed VBS-containing diets.

Table 2.3: Concentration of anti-nutritional factors (ANFs) of treated and untreated velvet bean seeds

Reference	Fathima *et al*. 2010	Mugendi *et al*. 2010	Vadivel and Biesalski, 2012	Gurumoorthi *et al*. 2013
Seed processing method	Oven heated at 80°C for 24 hours	Autoclaving at 121°C at 1 Kgf/cm for 30 minutes	Raw	Soaking in distilled water for 12 hours + microwave cooking with 2450MHz, 750 watts, and 230 volts for 10 minutes
Anti-nutritional factors (g/100g DM)				
L-Dopa	7.48	0.43	6.23	4.47
Tannins	0.3	0	3.49	0.1
Total free phenolic	4.4	0.44	8.65	0.74
Phytic acid	5.68	0.5	2.37	0.13

2.4.2. Tannins

In most of the explored raw VBS, a high level of tannins that ranges from 7.6 to 30 mg/kg DM was observed (Ravindran and Ravindran, 1988; Fathima *et al*., 2010). While on processed seeds tannins were observed to be 1.56 mg/kg DM (Ezeagu et al., 2003) and 2 to 3 mg/kg DM (Mugendi and Njagi, 2010). Tannins are known to be soluble in water, they are considered phenolic metabolites of plants with a molecular weight greater than 500 g/mol and with the ability to precipitate proteins from aqueous solution (Mehansho *et al*., 1987; Silanikove *et al*., 1994). They are secondary metabolites of plants, which are soluble in polar solution and because of their ability to precipitate protein; they are not considerably different from other polyphenolic compounds (Silanikove *et al*., 2001). Fifteen percent (15%) of herbaceous dicotyledonous plants and about 80% of woody plants contain tannins (Mahlo and Chauke, 2012). Bryant *et al*. (1992) also states that in some forages and feeds, tannins can occur at higher levels. Dube *et al*. (2001) indicates that condensed tannins bind with any other nutrient including dietary protein; hence reducing CP digestibility in animals. Makkar (2003) also observed reduced performance of animals fed high tanniferous feeds. To reduce tannin contents in the feed, polyethylene glycol (PEG) can be used to bind the harmful effects of polyphenolic compounds, such as tannins in livestock feeds, thus prevent the development of tannin-protein complexes (Ben Salem *et al*., 2005; Guerrero *et al*., 2012; Mokoboki *et al*., 2013).

2.4.3. Other Anti-Nutritional Factors

Although the concentrations of other anti-nutrients are usually too little to pose any significant threat in seeds, most of the food plants, more especially legume seeds, contain these anti-nutrients. In most instances, feeds derived from leguminous plants are usually processed and most toxicants are eliminated before consumption (Ezeagu *et al*., 2003).

Besides L-Dopa and tannins, VBS contain other anti-nutritional factors which include phytic acid, hydrocyanic acid, raffinose, trypsin inhibitor, oxalate (Iyayi and Taiwo, 2003; Ravindran and Ravindran, 1988; Ezeagu *et al*., 2003), verbascose, phenolics, and α-amylase inhibitor (Janardhanan *et al*., 2003; Bhat *et al*., 2007). The syntheses of phenolic compounds are somehow triggered by treating VBS by irradiation whereby it increases the activity of phenylalanine ammonia-lyase. Although phenolic compounds are also regarded as one of the major anti-nutrients, considerable interest has been recently shown in their possible antioxidant activities and potential health benefits (Bhat *et al*., 2007).

2.5. Processing techniques of velvet bean seeds

Processing methods of seeds were developed years ago to optimize nutrient utilization of many food grain legumes for both animals and humans (Sathyanarayana *et al*., 2016). Processing serves an additional purpose of purifying the VBS by decreasing the level of L-Dopa and other anti-nutritional factors to a safe level for successful digestion by animals without negatively affecting nutritional benefits of the seed (Bressani, 2002; Teixeira *et al*., 2003). Various methods that are used to process seeds in order to eliminate anti-nutritional factors include boiling (Teixeira *et al*., 2003), roasting (Randhir and Shetty, 2004), ensiling (Huisden *et al*., 2014), cooking and soaking in acid and alkaline solution (Wanjekeche, 2003).

2.5.1. Heat treatment

Boiling and roasting, among other heat treatment methods, are the most effective processes in reducing anti-nutritional factors present in VBS (Mugendi and Njagi, 2010). The majority of the toxic complexes in *Mucuna* beans are heat tolerant; therefore, for safe consumption by humans and other monogastric animals, cooking is considered the most common processing technique (Siddhuraju *et al*., 1996). Heat processing of VBS can be through roasting at 120°C for half an hour (Dossa *et al*., 1998; Randhir and Shetty, 2004), autoclaving for 30 min at

100°C until the seeds are brown and crispy (Del Carmen *et al*., 1999) and lastly, by grilling for 1 h after pre-soaking the seeds for 24 h and thereafter dehulling follows (Randhir and Shetty, 2004). Removing L-Dopa from grounded VBS of 1 mm particle size can be achieved by increasing water temperature from 40 to 66°C (Teixeira *et al*., 2003). Combining two processes, which is boiling for 1 hour and soaking using sodium bicarbonate for 24 h can reduce L-Dopa concentration content by 88% (Huisden, 2008). On the other hand Nyirenda *et al*. (2003) concluded that the principal method of reducing L-Dopa is through boiling. Roasting VBS enhances the flavour but reduces their nutritive value by lowering the levels of available amino acids (Bressani, 2002). While Mugendi and Njagi (2010) argued that processing increased the concentration of essential amino acids in VBS, it was also observed that roasting at 100°C did not affect the concentration of CP, however, autoclaving decreased CP content from 31.9 to 29.7 g/kg.

2.5.2. Fermentation

Fermentation is an anaerobic chemical process by bacteria or other microorganism in which molecules such as glucose is broken down to form gases such as carbon dioxide (Egounlety, 2003). To make food products such as tempe, the bacteria *Rhizopus oligosporus* has widely been used to ferment *Mucuna pruriens* (Egounlety, 2003). The study showed that the concentration of water-soluble protein increased from 1.22% to 19.42%, which directly indicates the increased proteolysis, but after 48 h, the process of fermentation did not have an effect on CP concentration. In another study, Mugendi and Njagi (2010) illustrated that fermenting *Mucuna* seeds at 32°C for 72 h increased the level of CP from 31.9 to 37.6%, and showed the reduction of L-Dopa levels by 73%; but increased the tannin content by 2%. Matenga *et al*. (2003) studied the ensiling of different levels of *Mucuna* seeds and maize. The results reported a decline in L-Dopa content of about 10% for a mixture of 100% *Mucuna* with 0% maize, and a decrease of 48% in a mixture of 30% *Mucuna* with 70% maize.

Therefore, the results clearly indicate that fermentation can be helpful for *Mucuna* purification strategy and its efficacy seemed to increase as more fermentable carbohydrates were supplied in the mixture.

2.5.3. Soaking in Acid and Alkaline solutions

In order to reach an extremely low L-Dopa content of about 0.01%, broken VBS should be added into $Ca(OH)_2$ solution (Diallo *et al*., 2002). However, probably due to conversion of L-Dopa to melanin the resulting mixture was blackish, therefore, further research was carried out to determine its suitability to humans. According to Mugendi and Njagi (2010), removal of L-Dopa at high pH (9.0) was sustained as 99.65% of L-Dopa was extracted. However, the bean samples and the extract turned black, implying chemical reaction of melanin from L-Dopa, rather than genuine extractor into water. Therefore the inactivation of L-Dopa in *Mucuna* seeds can be facilitated by alkaline conditions (Wanjekeche *et al*., 2003).

Levodopa is also very solvable when subjected to weak solutions of acetate (Teixeira *et al*., 2003). In fact, for quantifying L-Dopa, the process begins with extraction in hydrochloric acid using most traditional laboratory analytical methods (Bugamelli *et al*., 2011). In less than 8 h, the L-Dopa concentration in VBS of 1 mm particle size was reduced when soaked in acidified liquid of a pH of 3 (Teixeira *et al*., 2003). Lastly, Wanjekeche (2003) found that seeds soaked in acidic mixture are darker than raw beans but lighter than beans cooked in alkaline solution. This is also probably due to less melanin formation. The CP and crude lipid content of various processed seeds in different solutions were observed to be 317 to 332 and 49.3 to 50.2 g/kg, respectively and they were relatively higher than in raw seeds which was 301 and 43.4 g/kg, respectively (Siddhuraju and Becker, 2004).

2.6. Influence of *Mucuna pruriens* seed meal on broiler performance

In broiler production, the key indicators of performance are daily feed intake (DFI), daily body weight gain (DBWG), and feed conversion ratio (FCR). Therefore, in order to increase profitability and sustainability of a broiler production enterprise, growth performance should be a key in assessing the entire performance of the enterprise. Broiler performance enables the analysis of various factors such as genetics, the amount of feed supplied and health (Gallagher, 2017). Emenalon (2004) reported that when unprocessed VBS were fed to laying hens, the performance was compromised, toasting and boiling was found to effectively remove the causative poisonous substance. Tuleun *et al*. (2011) concluded that based on comparing the results obtained from growth performance and carcass quality characteristics, it can be reasoned that dietary inclusion of not more than 20% of cooked and roasted VBS can be used effectively as a plant protein source in a broiler diet without decreasing the performance of broilers. Since feeding velvet bean seed meal to broilers has been widely investigated (Del Carmen *et al*., 1999; Iyayi and Taiwo, 2003; Nyirenda *et al*., 2003; Jayaweera *et al*., 2007; Ani, 2008; Vadivel and Pugalenthi, 2010; and Mangwiro *et al*., 2013); therefore, Table 2.4 illustrates some of the growth performance parameters of broilers fed VBSM as reported from literature.

Table 2.4: Feed intake and growth performance values of broilers fed diets with velvet bean seed meal

Growth performance parameters	**Range**	**Source**
Average daily feed intake (g/bird)	79.52 - 118.8	Del Carmen *et al.* 1999; Jayaweera *et al.* 2007; Tuleun *et al.* 2009; Vadivel *et al.* 2010
Final body weight (g/bird)	1525 - 1852	Jayaweera *et al.* 2007
Average daily gain (g/bird)	26.32 - 54.8	Del Carmen *et al.* 1999; Jayaweera *et al.* 2007; Tuleun *et al.* 2009; Vadivel *et al.* 2010
Feed conversion ratio	1.67 - 3.23	Del Carmen *et al.* 1999; Jayaweera *et al.* 2007; Tuleun *et al.* 2009; Vadivel *et al.* 2010
Dressing percentage (%)	67 - 76	Del Carmen *et al.* 1999; Jayaweera *et al.* 2007;
Mortality (%)	0.2 - 5.6	Del Carmen *et al.* 1999

2.6.1. Feed intake

The nutrient intake of animals is influenced by both the nutrient composition of the diet and the feed intake in general. Therefore, Jayaweera *et al*. (2007) states that feed intake was not affected by velvet bean. No difference was also observed on feed intake of pigs fed different processed (soaked in potassium carbonate, toasted and boiled) and raw velvet bean seed meal (Onigemo and Anjola, 2013). In an observation by Del Carmen *et al.* (1999), feed intake by broilers declined only with 30% inclusion of raw velvet bean seed meal. However, on a similar study, feed intake was reduced at 20% and 10% inclusion levels of cooked and toasted velvet meals, respectively. Hence, in the study by Emenalom and Udedibie (1998); and Mugendi *et al*. (2010), it can be concluded that the differences in feed intake may be due to the level of seed treatment and the processing techniques.

2.6.2. Feed conversion efficiency

In layers, Tuleun *et al*. (2008) found a difference in feed conversion efficiency in terms of the amount of feed consumed and weight of egg produced between toasted, unprocessed, and cooked velvet bean diets. In a study conducted by Jayaweera *et al*. (2007) it was observed that 20 and 25% velvet bean inclusion in broiler diets increased feed conversion ratio by 23 and 17% respectively. Similar results were also observed by Del Carmen *et al.* (1999), whereby feed conversion efficiency of broilers decreased when fed a diet with 20 and 30% velvet bean inclusion. Diets with different processed VBSM (cracked, soaked and cooked) performed better in terms of FCE than the unprocessed VBSM diet (Emenalom *et al*., 2004).

2.6.3. Body weight gain

Carew *et al*. (2003) concluded that the body weight gain of broilers decreased after feeding raw velvet bean seeds. While Del Carmen *et al.* (1999) observes that inclusion of 10% heated velvet bean seeds permitted better growth than unprocessed bean and by the end of the experiment (day 42), growth was not much different from the control diet. On the other hand

Jayaweera *et al*. (2007) observes that not only raw seeds can decrease body weight gain but 20-25% inclusion of velvet bean seeds into a diet decreases weight gain. Therefore, from that state, it is suggested that some anorexic factors in unprocessed velvet bean might not be heat labile, but with the combination of different processing methods, these anorexic factors may be denatured (Carew *et al*., 2003; Ani, 2008). Table 2.4 below shows feed intake and some of the growth performance parameters of broilers fed VBSM respectively as reported from literature. To improve broiler performance, the amount of VBSM included should be taken into consideration, as high levels reduces FI, BWG, and increases FCR. From literature it has been demonstrated that less than 20% inclusion of VBSM in the diets does not have any negative effect on growth performance of broilers.

2.7. Influence of velvet bean seed meal on broiler meat quality parameters

2.7.1. Meat pH

Meat pH has a wide effect on the water retaining properties and colour of the meat, as well as the susceptibility of meat to bacterial spoilage and its shelf life (Petracci *et al*., 2015). Temperature and pH are two of the main factors that contribute to protein denaturation and the development of PSE (pale soft and exudative) in white meat (Barbut *et al*., 2008; Lammers *et al*., 2007). This is a result of the production of lactic acid from quick breakdown of glycogen after slaughter. Muchenje *et al*. (2009) stated that meat quality is influenced largely by the decrease of pH in the muscles after slaughter. Over 24 h post slaughter, the pH in the muscle decreases from approximately 7.0 - 7.2 down to a range of 5.5 - 5.7 for normal meat (Hambrecht *et al*., 2004). However, if the meat pH decreases to a pH of 5.5 - 5.7 in an hour or less immediately after slaughter or below normal ultimate pH, the muscle will look pale, soft, and exudative (PSE). In such instances, the meat will be regarded as being of poor quality and will not be good for consumers (Desai *et al*., 2016). Factors such as colour and the ability of the meat to retain water is influenced by the variation in ultimate pH (Fletcher, 2002). Low ultimate pH results in meat proteins having a lighter colour and decreased water-holding capacity. In opposition, a higher ultimate pH will result in a darker colour and less drip loss (Yusop *et al*., 2010). Ultimate pH also affects eating quality characteristics such as juiciness, tenderness, and flavour. There are a few studies that have been conducted on meat pH from livestock fed VBSM, where the ultimate meat pH varied between 5.7 - 5.76 in guinea fowl fed diets with raw, cooked, and toasted VBSM (Dahouda *et al*., 2009). Beside feeding velvet bean seed meal, Laudadio and Tufarelli (2010) observed that the variables related to chicken meat pH after 24 h post-mortem were not influenced by the dietary treatment containing de-hulled peas (Laudadio and Tufarelli, 2010).

2.7.2. Meat colour

The colour of meat is one of the characteristics that can easily be noted by consumers, more especially in boneless meat parts, and it is among the indicators of meat quality **(**Kennedy *et al*., 2004). The concentration of the meat pigments, essentially myoglobin, and the chemical state of myoglobin determines the colour of the meat (Rosenvold and Anderson, 2005). The meat colour can be measured by tricolorometric measurements such as the Laboratory systems, whereby L*, a* and b* are the colour coordinates indicating lightness, redness and yellowness, respectively, in the defined colour room (Muchenje *et al*., 2009; Pathare *et al*., 2013). The Lab-systems have been found to be appropriate to characterise meat colour and especially the a-value seems to correlate well to sensory properties of the meat (Rosenvold and Anderson, 2005). The colour of the meat can be affected by several factors, like the age at slaughter, pre-slaughter stress, amount of enzymes in the diet, and even the activities undertaken by the animal. For instance, the protein that is liable for the distribution of the reddish appearance in muscle (myoglobin) does not circulate well in the blood but is fixed in the tissue cells and is purplish in colour (Muchenje *et al.*, 2009).

Dark firm and dry (DFD) meat is of poor quality as it is characterised by poor colour and taste and hence the dark coloured meat is less satisfactory to the consumer. Due to the abnormal high pH value the meat tends to have a shorter shelf life, which is highly conducive to bacterial growth (Priolo *et al*., 2001). Hence, Zhang *et al*. (2005) highlighted that high pH meat had lower a* (redness), L* (lightness) and b* (yellowness). The inclusion of VBSM in broiler diets did not have an effect on the colour and the overall acceptability of the meat. According to Wood *et al*. (1995), this may be due to the insignificant influence of the processing methods on the fatty acid composition in the diet of broiler chickens. Recently, there is no evidence on studies pertaining the analysis of meat colour from chicken meats fed VBSM. However, in terms of other related legume seeds, Laudadio and Tufarelli (2010) observed that the inclusion of peas did not have an effect on breast and drumstick yellowness from broilers. Furthermore, the L* of breast and drumstick values were in the normal range of 6.12-6.09. According to Laudadio *et al*. (2011)'s observation, breast and drumstick meat from broilers fed the faba bean diets had lower L* (44.62), and higher a* (9.61) and b* (2.67) values when compared to broilers fed the soybean meal diets (46.77, 8.87, and 2.01) respectively.

2.7.3. Meat tenderness and Flavour

Tenderness is well known as the toughness and softness of the meat (Koohmaraie *et al*., 2002), thus, the tougher the meat, the more force is required to cut it, and that is known as the Warner–Bratzler shear force (WBSF) test (Muchenje *et al*., 2009). Meat tenderness is very important to meat quality and consumer acceptance, although it is a trait whose prediction is difficult. It relies on simplicity of chewing that is contributed by many factors. The tenderness of the meat is influenced by factors such as sarcomere shortening during rigor mortis, the amount and solubility of connective tissues, the rate of post mortem energy metabolism, post mortem proteolysis of myofibrillar proteins, and marbling (Warner *et al*., 2010). Among these factors, the fibrous nature of muscle contributes to chewing resistance (Gerrard and Grant, 2003). What leads to more strength in muscle is the muscle fibres and they eventually decrease tenderness of the meat, because many myofibrils are arranged in register across the fibres (Dinh, 2008). Based on thickness and organization of fibre bundles, the connective tissues are divided into two main categories: which are loose and dense connective tissues **(**Dinh, 2008; Kumka and Bonar, 2012). Satisfaction of consumers mostly depends on the tenderness and flavour of the meat after cooking (Glitsch, 2000). Thus, meat sensory features are important in determining the eating pleasure of consumers and can influence decisions on future purchasing intent and consumption.

Among sensory traits of meat, flavour and tenderness are very vital in determining quality of meat cuts (Thu 2006). Flavour and tenderness rely mostly on meat texture and composition. It also includes the sensory appeal of meat such as palatability, especially in relation to the amount and type of fat and other fat components (Hwang and Joo, 2017). In the few studies conducted on broilers, the inclusion of VBSM had no effect on breast tenderness, juiciness and flavour (Adzitey *et al*., 2010). On other related legume seeds that were fed to broilers, Milczarek *et al*. (2016), observed that the inclusion of faba beans to broiler feeds had an effect on texture properties whereby the meat was found to be tender.

2.7.4. Cooking loss, dripping loss and quality

By definition, cooking loss is the amount of drop or decrease of meat during cooking (Tornberg, 2005). The loss known as the volatile losses and as drippings occurs during the cooking of the meat. Evaporation of water is regarded as the greater part of the volatile loss (King and Whyte, 2006). The evaporation may include volatile substances from the volatile aromatic substances and the decomposition of fat. The drippings from cooked meat include nitrogenous and non-nitrogenous extractives, fats, salts and water (Adzitey *et al*., 2010). Many factors affect cooking loss, which include pH of the muscle, final cooking temperature, and the method of cooking (King and Whyte, 2006). There is a direct proportion between the amount of water that is driven from the muscle as well as the amount of lipid or fat that is liquefied and allowed to excrete, and the final cooking temperature (Adzitey *et al*., 2010). Jama *et al*. (2008) states that ageing also affects cooking loss as it was observed that, as ageing increased lower cooking losses were detected. Yu *et al*. (2005) also showed that the temperature during defrosting affects dripping loss where leg and breast meat samples defrosted at 18°C had higher drip loss compared to meat defrosted at 0°C or 2°C. According to Ali *et al*. (2016), drip loss increased and water-holding capacity decreased when the thawing rate was increased. Jama *et al*. (2008) recommended that each component of cooking loss has to be determined when evaluating for cooking loss as a processing quality trait and meat eating. In the study where Guinea Fowl were fed raw, cooked, and toasted VBSM, drip loss and cooking loss values were found to be lower than the range of 2.31-3.87 and 11.2-13.42 respectively (Dahouda *et al*., 2009). Currently there is no evidence on studies where meat quality attributes of broilers fed VBSM was studied. Therefore, there is a need for further research on these specific aspects.

2.8. Velvet bean seed meal on fatty acids profile of chicken meat

Fatty acids are in the form of saturated fatty acids (SFA) and unsaturated fatty acids (USFA) (Nantapo *et al*., 2015; NHS, 2017). In general, fatty acids are categorised based on the number of double bond between carbon atoms. Monounsaturated fatty acids have one double bond and poly-unsaturated fatty acids have two or more double bonds, whereas saturated fatty acids have no double bond (NHS, 2017). Free fatty acids and trans-fatty acids are included as being the other classes of fatty acids (Williams, 2000), even macro and micro-constituents of the Mediterranean diet and polar lipids are included as classes of fatty acids (Nasopoulou *et al*., 2011). The omega-3 and omega-6 are the two main types of polyunsaturated fats. These are essential fats that should be obtained from broiler meat. They have important benefits for consumer's heart, brain and metabolism (NHS, 2017).

Fats from chicken meat include both saturated fatty acids and monounsaturated fatty acids. In lamb muscle and fat, traces of Butyric (4:0) were identified; it was, however, absent in chicken meat (Woods and Fearon, 2009). Oleic (C18:1), stearic (C18:0) and palmitic (C16:0) acids are the most abundant fatty acids in chicken breasts (De Marchi *et al*., 2011). Into some extent, more unsaturated fatty acids of about 10 to 15% of total fatty acids can be found in poultry meat (Ovaskainen *et al*., 2001). On the other hand, Belury (2002) found that about 1 to 2% of total fatty acids across all types of meat is Trans-fatty acids. When comparing to beef and mutton, chicken meat is a better source of polyunsaturated fatty acids (Jahan *et al*., 2004).

Age of the animal, species, and part of the carcass influence fat content in meat and meat products (Valsta *et al*., 2005). Animal feeding also affects the fat composition and fat content (Bolte *et al*., 2002). And also superior health properties are linked with high polyunsaturated fatty acids content (Hugo and Roodt, 2007). The effect of feeding VBSM on fatty acid profiles of broiler chicken meat is not yet understood, thus further research is needed on this aspects. Nevertheless few studies has been carried out in related legume seeds, where in broilers fed faba beans, more linoleic acid, linolenic acid, and more PUFAs were found in drumstick meat (Milczarek *et al*., 2016). Tufarelli and Laudadio (2015) also observed an increased level of linoleic acid and α-linolenic in the muscles of broilers fed faba bean. While Laudadio *et al*. (2011) reported that the total n-3 fatty acids were higher in drumstick meat of broilers fed faba bean diet when compared with control soybean diet and higher levels of palmitic acid led to an increase in total saturated fatty acids in the muscles.

2.9. Chicken fatty acids and health issues

The choice of food is mainly determined by the income of an individual, and it thus affects the types and quantity of nutrients and substances that individuals consume. More scientific evidence on food consumption and related health risk has improved consumer awareness of dietary health issues and also influenced public opinion and media on diet and health issues (Jiménez-Colmenero *et al.*, 2001). A high consumption of various types of fats has harmful effects on human health (Mensink *et al*., 2016). Excessive weight gain is mainly caused by high levels of fat in the human diet, resulting in a high risk of diseases such as high blood pressure, heart diseases, and diabetes (WHO, 2016). In addition, NHS (2017) states that unwanted fats by the human body are a predictor of heart disease, diabetes, insulin resistance and some cancers. The grade of unsaturated fatty acids has also been identified as a potential risk on health because it has an effect on the type of cholesterol found in meat (Navidshad *et al*., 2015). Generally, saturated fats have high levels of low-density lipoprotein (LDL)-cholesterol and they result in the risk of heart diseases, except saturated fatty acids because they have 18 or more carbon atom bond in their chain since these chains have higher levels of high-density lipoprotein (HDL)-cholesterol (Verbeke *et al*., 1999; Pottel *et al*., 2014). High intake of chicken that is rich in n-3 fatty acid result in prevention of chronic diseases (Natalie *et al*., 2009).

2.10. Conclusion

The objective of this review was to summarise the current research on the use of VBSM as a protein source in broiler chicken feed, and its challenges and benefits on livestock performance and meat quality. Therefore, literature confirmed that VBS can be included into a meal as an alternative source of protein for mono-gastric animals, but its inclusion level should not exceed 20% in the diet. The main challenge on using the seeds is that they possess toxic amino acids called levodopa/ L-dopa and other anti-nutrients. However, heat treatment, fermentation and acid and base soaking, among other processing techniques were shown to be effective on eliminating these anti-nutrients. Currently, there are a high number of studies conducted on feeding VBS to broilers. However, the majority of researchers have focused on broiler performance and there is very little information on its effect on the quality of meat and fatty acids produced by broilers fed VBSM, thus there is a need for further research on these aspects.

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Chapter 3: The effect of feeding varying levels of heat processed velvet bean (*Mucuna pruriens*) seed meal on feed intake and growth performance of broilers

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Abstract

In this study, the feed intake and growth performance of broilers fed varying levels of heat processed velvet bean seed meal (VBSM) were determined. It is considered as a viable source of dietary proteins due to its reasonable high protein concentration of 33.4%. South African production levels do not meet the current demand of soybean, hence majority is imported. Their scarcity results in increased price of broiler feed and eventually affect the growth of the broiler industry. Therefore there is a need to explore velvet bean which can grow in poorly fertilised and low rainfall areas. The study was conducted at Fort Cox Agriculture and Forestry Training Institute. Four diets were formulated to be iso-caloric and iso-nitrogenous comprising velvet beans at 0, 10, 15 and 20 % (T1, T2, T3 and T4), respectively. The broilers were reared for 42 days with 35 days on experimental diets. Average feed intake, body weight gain, feed conversion ratio, mortality and final body weight were recorded. The data was analysed using General Linear Model (GLM) procedure of SAS (SAS, 2006), mean separation was done using LSD test option of SAS. Birds on diet T1 had higher ($P < 0.05$) BWG than diet T2, T3 and T4. Broilers that were given T1 diet had higher ($P < 0.05$) DFI than those on diets T2, T3 and T4. Daily feed intake (DFI) of broilers was high ($P < 0.05$) in control diet compared to other treatments in both Phase 1 and Phase 2 feedings. Broilers fed T1 diet had higher ($P < 0.05$) ADG compared to those fed diets with VBSM. Average daily gain (ADG) from broilers fed T1 diet was higher ($P < 0.05$) compared to other treatments in both Phase 1 and Phase 2. Feed conversion ratio (FCR) were not influenced by diets ($P > 0.05$). Broilers offed diet T1 were heavier ($P < 0.05$) than those fed diets T2, T3 and T4. It can be concluded that the VBSM has a negative effect on feed intake, growth performance and mortality of broilers, although there is no effect on FCR.

Key Words: Anti-nutritional factors, broiler, feed intake, plant protein source.

3.1. Introduction

The key objective of broiler producers is to produce meat that will satisfy consumer's preference at a low feeding cost. In order to maximise broiler performance and profit, good nutritional feeds with adequate protein, energy and other nutrients are required to meet the nutrient requirements of broilers for growth (Skinner *et al*., 1992; Beski *et al*., 2015). Besides promoting growth, nutrition influences the end product such as quality of meat (Liu *et al*., 2012). Legume plants and seeds traditionally provide the major portion of protein required by animals (Carew *et al*., 2003; Balogun and Olatidoye, 2012). However, due to their deficit in one or more amino acids, plant proteins are usually formulated with synthetic amino acids and other protein sources such as processed fish meal (Cotton *et al*., 2016; Bajjalieh, 2017; and Hansen, 2017).

In South Africa, both commercial and smallholder broiler enterprises are growing, due to general increase in price of other meat protein sources, which results in increased demand for broiler feeds. Soybean meal and sunflower meal are currently the main usable protein sources in broiler feed, but they are failing to meet increasing demand (Chisoro *et al*., 2016). Soybean is expensive. In addition, the South African production levels do not meet the current demand, hence in Sub-Saharan Africa, 72% of soybean oilcake is imported in South Africa (Grain SA, 2016). Their scarcity results in increased price of broiler feed and eventually affect the growth of the broiler industry. Exploring the potential use of underutilised indigenous legumes such as velvet bean would assist the smallholder farmers and the broiler industry. Indigenous leguminous plants such as velvet bean can be planted in poor soils and low rainfall areas and are usually intercropped with maize in communal smallholder farming systems (Mureithi *et al*., 2003; Jiri and Mafongoya, 2018).

Velvet bean`s (*Mucuna pruriens*) foliage is frequently fed to grazing animals and the beans are sometimes eaten by humans and animals such as poultry and pigs (Camara *et al*., 2003;

Muinga *et al.*, 2003). There is very low or no interest in *Mucuna* cultivation due to its bad utility by animals, and the lack of market for the seeds in Southern Africa (Eilitta and Carskey, 2003). Furthermore, the use of VBS either as human food or as animal feed is limited due to anti-nutritional components that naturally exist within their structures, which can adversely affect the quality of the protein for utilisation by the animal (Bhat *et al*., 2007; Akande *et al*., 2010). These anti-nutritional factors include L-dopa (3, 4 Dihydroxy-L-phenylalanine), which is a toxic amino acid and a neurotoxic agent (Del Carmen *et al*., 1999; Ani, 2008; Mugendi *et al*., 2010), trypsin inhibitors, tannins, phenolic, phytic acids, and oxalate (Ravindran and Ravindran, 1988; Iyayi and Taiwo, 2003; Mugendi *et al*., 2010). The feed potential of *Mucuna pruriens* can be enhanced by reducing these anti-nutritional factors to safe levels either by boiling and roasting or by fermentation (Matenga *et al*., 2003; Teixeira *et al*., 2003; Randhir and Shetty, 2004; Mugendi *et al*., 2010). It was hypothesised that feeding varying inclusion levels of heat processed VBSM does not affect feed intake and growth performance of broilers. The objective of this study was to investigate the effect of feeding varying levels of heat processed VBSM on feed intake and growth performance of broilers.

3.2. Materials and methods

3.2.1. Ethical clearance

Ethical clearance to carry out the study was applied for, and approved, by the University of Fort Hare's Research ethics committee (UREC). The certificate clearance number is GAJ041AMTH01.

3.2.2. Description of study site

The study was conducted at Fort Cox Agriculture and Forestry Training Institute, situated in Raymond Mhlaba Local Municipality under Amatole District, in the Eastern Cape Province of South Africa. The geographical location of Fort Cox Training Institute is 32°43′48″S and 27°1′32″E. The annual rainfall is about 412 mm, of which the most rainfall is received in autumn. The midday temperature ranges between 8°C in June to 29°C in February. Altitude is 508 meters above sea level. The experiment was carried out under a deep litter system with 5-8 cm depth saw dust in the poultry house. The ventilation was manually controlled by opening the curtains every morning. The temperature of the house was controlled using electric heaters and measured by a thermometer in order to maintain the temperature at 25-36 °C for the first three weeks of the experiment.

3.2.3. Feed preparation and formulation

Raw velvet bean seeds were purchased from McDonald Seed Company in Pietermaritzburg. The seeds were processed by heating at 130°C for 30 min (Carew *et al*., 2003) using memmert laboratory oven. They were then ground using a laboratory mill to pass through a 1 mm screen and stored in bags at room temperature. The milled samples were subjected to proximate analysis to obtain the nutrient content (Table 3.1). Moisture content was determined by obtaining the difference in weight of dried and fresh samples. The ether extract was determined using Soxhlet extraction method while crude protein was determined using Kjedahl method by multiplying the nitrogen content with 6.25. In addition macro (Calcium, Phosphorus, Potassium, and Magnesium) and micro (Iron, Copper, Zinc, and Manganese) minerals were analyzed using an Atomic Absorption Spectrophotometer. Nitrogen Free Extract (NFE) was calculated as NFE = DM - (Crude protein + Crude fiber + Ether extract + Ash) in terms of percentage. The metabolisable energy (ME) in kcal/kg of *Mucuna pruriens* seed meal was calculated using the method by Pauzenga (1985), were ME = 35 x CP % + 81.8 x EE% + 35.5 x NFE%. Total phenolic and condensed tannins contents of the seeds were determined as described by Idris *et al*. (2017) using water extracts. The oxalate and Phytic acid content of the seed was determined as described by Unuofin *et al*. (2017) using water extracts (Table 3.2). Diets were formulated on FORMAT New Century Single-mix 250 version Formulation Software to be iso-caloric and iso-nitrogenous to meet the broiler nutrient requirements (NRC, 1994) (Table 3.3). All the formulated experimental diets were subjected to proximate analysis to obtain the moisture, crude protein, ether extract, crude fiber, ash and mineral composition according to Association of Official Analytical Chemists (AOAC, 2000). The neutral detergent fibre and acid detergent fibre contents were determined using an ANKOM200/220 fibre analyser (ANKOM Technology, Fairport, NY, USA) by the methods described by Robertson and Van Soest (1981). (Table 3.4).

Table 3.1: Chemical composition of heat processed velvet bean seed powder

Chemical components (%)	Composition
Moisture	7.5
Crude Protein (CP)	33.4
Crude Fibre (CF)	7.8
Ether extract (EE)	8.4
Ash	3.3
Nitrogen free extract (NFE)	39.6
Total digestible nutrients (TDN)	77.4
Metabolisable Energy (kcal/kg)	3261.92
Major minerals (%)	
Calcium (Ca)	0.07
Phosphorus (P)	0.70
Potassium (K)	0.19
Magnesium (Mg)	0.09
Trace minerals (mg/kg)	
Iron (Fe)	126
Cupper (Cu)	13
Zinc (Zn)	17
Manganese (Mn)	40

Table 3.2: Analysed anti-nutritional factors (ANFs) of heat processed velvet bean seed powder

Anti-nutrients	**Levels**
Condensed tannins	61.95 (mg CE/g)
Total free phenolic	1912.54 (mg GAE/g)
Phytic acid	19.96 (%)
Oxalate	3.81 (mg)

mg CE/g- milligram of catechin equivalent, mg GAE/g- milligram in gallic acid equivalent per grams, %- percentage, mg- milligram.

Table 3.3: Ingredients compositions of experimental diets

Ingredients	Starter				Finisher			
	T1	T2	T3	T4	T1	T2	T3	T4
Yellow maize	61.00	60	53.66	53.68	70	65	58.39	52.53
Soya Oil cake 47	29.09	21.83	23.89	13.86	20.63	15.80	11.73	12.53
Full Fat Soya	4.07	4.63	3.00	6.14	2.99	4.1	9.82	9.90
Velvet bean seed meal	0	10	15	20	0	10	15	20
Canola Oil	2.00	2.00	2.00	2.00	2.00	2.00	2.00	2.00
Limestone Fine	1.91	0.13	0.00	1.50	1.38	1.43	1.34	1.32
Mono calcium phosphate	0.59	0.00	0.84	0.85	0.65	0.47	0.50	0.51
Sodium Bicarbonate	0.09	0.14	0.31	0.25	0.61	0.06	0.09	0.08
Salt fine	0.38	0.34	0.22	0.27	0.02	0.35	0.33	0.34
Lysine	0.20	0.30	0.41	0.52	1.20	0.17	0.17	0.16
Methionine	0.18	0.15	0.18	0.20	0.08	0.14	0.15	0.15
Threonine	0.00	0.00	0.00	0.22	0.00	0.03	0.03	0.03
Tryptophan	0.00	0.00	0.01	0.03	0.00	0.00	0.00	0.00
Axtra Phy broiler enzyme blend 600 px	0.06	0.06	0.06	0.06	0.06	0.06	0.06	0.06
Aviax plus	0.05	0.05	0.05	0.05	0.05	0.05	0.05	0.05
Surmax	0.04	0.04	0.04	0.04	0.04	0.04	0.04	0.04
Min-Vit Premix	0.34	0.33	0.33	0.33	0.29	0.30	0.30	0.30
Total (%)	100	100	100	100	100	100	100	100

Table 3.4: Analysed nutrient composition of the experimental diets

Nutrients composition	Starter				Finisher			
	T1	T2	T3	T4	T1	T2	T3	T4
Proximate analysis								
Moisture (%)	9.2	8.6	9.5	9.1	10.4	10.8	10.2	10.1
Crude protein (%)	23.2	22.7	23.2	21.4	19.2	19.5	20.0	20.4
Ether extract (%)	7.1	7.2	8.4	7.1	7.1	6.7	8.3	2.1
Acid Detergent Fibre (%)	3.6	4.9	3.7	4.1	2.7	3.7	3.3	3.3
Neutral Detergent Fibre (%)	19.9	25.9	20.1	21.3	33.7	42.8	20.6	29.3
Ash (%)	6.4	3.5	4.2	5.5	5.1	4.9	4.8	5.4
Metabolisable Energy (MJ/kg)	11.2	11.0	10.8	10.6	11.2	10.7	11.3	10.8
Macro minerals (%)								
Ca	0.8	0.2	0.3	0.9	0.9	0.8	0.7	0.8
P	0.5	0.4	0.6	0.6	0.5	0.5	0.5	0.5
K	1.0	1.0	1.1	1.0	0.8	0.9	0.9	1.0
Na	0.5	0.1	0.1	0.1	0.1	0.2	0.1	0.2
Mg	0.2	0.2	0.2	0.2	0.1	0.2	0.1	0.1
Micro minerals (mg/kg)								
Fe	235	149	187	231	170	242	190	230
Zn	98	110	132	115	52	173	109	124
Cu	8	11	14	12	6	12	10	13
Mn	102	125	146	120	40	126	110	131

T1, T2, T3 and T4 contained varying inclusion levels of *Mucuna pruriens* seed meal at 0, 10, 15 and 20% dry matter, respectively.

3.2.4. Experimental design, broiler feeding and management

A total of 112 *Cobb* unsexed day-old chicks weighing 45.2 g on average were purchased from Buffalo chicks in Berlin, East London. On arrival all chicks were placed in one floor pen (2×2 m) with 4 brooders, and they were introduced to a commercial starter feed for seven days for adaptation to the environment. Four dietary treatments with different inclusion levels (0, 10, 15, and 20%) of VBSM were used. The seven day-old chicks were randomly selected and placed on different treatment diets. Each treatment was replicated four times with seven birds per replicate and placed in a 1×2 m floor pen. Since there is not much difference in nutritional composition between starter and grower diets (Saleh *et al*., 1997), the birds were fed starter (7 to 28 days) and finisher (29 to 42 days) diets containing four concentrations of VBSM. Feed and water were offered *ad libitum* throughout and electric light was also offered throughout the experiment. All the birds were housed in a low-cost housing unit, where ventilation, humidity and temperatures were not artificially controlled.

3.2.5. Broiler vaccination

On arrival, all chicks were offered Virbac-Stresspac in their drinking water for two days. Virbac-Stresspac provided Vitamins and electrolytes. On day seven, after dividing the chicks into replicate pens, they were supplied with Triple sulfa in drinking water, triple sulfa is an anti-protozoal and provides electrolytes. On day 10, they were supplied with MA5+CLONE30 dissolved in rainwater, a vaccine for Newcastle Disease and Bronchitis. On day 14, broilers were offered Gumboro D78 dissolved in rainwater for immunising broilers against Infectious Bursal Disease (Gumboro). On day 21 Tylan was supplied in tap water to broilers, tylan is an anti-mycomplasmal. Then on day 23, ND Clone30 was offered in rainwater to broilers for the prevention of Newcastle disease.

3.2.6. Slaughter procedure

Birds were slaughtered at day 42. Animal slaughter and dressing was done following usual commercial procedures at Fort Cox poultry abattoir. Eighty birds were randomly selected, 20 birds per treatment were fasted for five hours with water offered *ad libitum*. The birds were individually stunned on the head using electrical stunning method with 70 volts, and frequency of 50 Hz. While unconscious, a single cut to the base of the throat of birds was performed by a trained worker using a sharp knife. After slaughter, evisceration was done where feathers, internal organs, head and feet were removed.

3.2.7. Broiler performance data collection

All the chicks were weighed on placement. The feed consumed by all the birds in all replicates per treatment was recorded weekly. Body weights (BW) were recorded weekly by weighing each bird in each replicate. Feed was supplied continuously by constantly weighing the refuse from the troughs. Feed wastage was minimized by filling the troughs to about three quarter full. Feed intake (FI) was taken weekly in all replicate per treatment by subtracting the amount of feed left from the amount of feed supplied and dividing with the number of birds in each treatment to obtain the average feed intake per bird, feed intake was divided by seven days in order to get daily feed intake (DFI). Body weight gain (BWG) was taken on weekly basis for each treatment and dividing with the number of birds in each treatment to obtain average body weight gain, BWG was divided by number of days in order to get daily weight gain (DWG). Feed conversion ratio (FCR) was calculated by dividing FI by BW. The feed intake (FI), bodyweight gain (BWG), and feed conversion ratio (FCR) was calculated as follows:

$$\text{Average feed intake} = \frac{\text{Total feed given (g)} - \text{Total feed leftover (g)}}{\text{Experimental perod (days)}}$$

$$\text{Average body weight gain} = \frac{\text{Final body weight (g)} - \text{Initial body weight (g)}}{\text{Experimental periods (days)}}$$

$$\text{Feed convesion ratio} = \frac{\text{Feed intake (g)}}{\text{Body weight gain (g)}}$$

3.2.8. Statistical analysis

The effect of dietary treatments on FI, BWG, ADFI, ADG, FCR and Mortality of broiler chickens was analysed using the analysis of variance (ANOVA), using General Linear Model (GLM) procedure of SAS (SAS, 2006). Mean separation was done using LSD test option of SAS (2006). The statistical model used was:

$$Y_{ij} = \mu + \alpha_i + \varepsilon_{ij}$$

Where: Y_{ij} = response variables (ADFI, ADG, FCR and Mortality),

μ = the common mean,

α_i = the effect of dietary treatment (T1, T2, T3, and T4), and

ε_{ij} = random error

3.3. Results

3.3.1. Body weight gain

Body weight gain of broilers fed 0% velvet bean diet was higher ($P < 0.05$) than those fed diets with 10, 15 and 20% velvet bean seed (Table3.6). Body weight gain of broilers fed control diet was also higher ($P < 0.05$) in both Phase 1 and Phase 2 compared to those fed diets with VBSM (Figure 3.1).

3.3.2. Average daily feed intake

Broilers on diet T1 had higher ($P < 0.05$) feed intake than those on diets with 10, 15 and 20% VBSM inclusion levels. However, no significant difference ($P > 0.05$) was observed in daily feed intake among broilers fed diets with 10, 15 and 20 VBSM (Table 3.6). Daily feed intake of broilers was higher ($P < 0.05$) in control diet compared to other treatments during both phase 1 and phase 2 feeding (Figure 3.2).

3.3.3. Average daily gain

Broilers fed 0% VBSM diets had higher ($P < 0.05$) average daily gain compared to those fed VBSM-containing diets (Table 3.6). Daily gain from broilers fed 0% velvet bean diet was higher ($P < 0.05$) compared to other treatments in both Phase 1 and Phase 2 (Figure 3.3).

3.3.4. Feed conversion ratio

Feed conversion ratios were not influenced by diets ($P > 0.05$) (Table 3.6). A non-significant difference ($P > 0.05$) was also observed across all treatments in both Phases 1 and 2 (Figure 3.4).

3.3.5. Mortality

Higher ($P < 0.05$) death incidences were observed on broilers fed a diet T1 than those fed diets T2, T3 and T4 (Table 3.6). In Phase 1 a high ($P < 0.05$) mortality was observed in broilers fed a control diet while no mortality was observed in other treatments. No significant difference ($P > 0.05$) in mortality was seen across all treatments on Phase 2 however, T1 had numerically high mortality of 7.1% (Table 3.8).

Table 3.5: Least square mean of body weight gain, average daily feed intake, average daily gain, feed conversion ratio and mortality of broilers fed varying inclusion levels of velvet bean seed meal for 42 days period

Treatment	**Parameter**				
	BWG (g)	ADFI (g)	ADG (g)	FCR	Mortality (%)
T1	1521.85^{a}	134.95^{a}	67.98^{a}	1.98	28.6^{a}
T2	1176.07^{b}	105.42^{b}	53.45^{b}	2.00	0^{b}
T3	1104.65^{b}	109.02^{b}	51.59^{b}	2.16	0^{b}
T4	1200.11^{b}	110.91^{b}	54.85^{b}	2.00	2^{b}
SEM	39.842	4.391	1.780	0.081	1.882
P-value	<0.0001	<0.0001	<0.0001	0.3241	<0.0001

^{ab} Means in the same column with different superscripts differ significantly ($P < 0.05$). SEM = Standard error of means, BWG - Body weight gain, ADFI = Average daily feed intake, ADG = Average daily gain, FCR = Feed conversion ratio, % = percentage, and g = grams. T1 is a control, T2, T3, and T4 contains 10%, 15% and 20% *Mucuna pruriens* seed meal as per DM intake, respectively.

3.3.6. Final weight

Broilers offered diet with 0% VBSM were heavier ($P < 0.05$) than those fed diets with 10, 15 and 20 % VBSM (Table 3.7).

Table 3.6: Least square mean of initial (7 days old) and final (42 days old) body weight of broilers fed varying inclusion levels of velvet bean seed meal

Treatment	Parameter	
	Initial weight (g)	Final Weight (g)
T1	156.03	2438.80^{a}
T2	151.05	1915.15^{b}
T3	150.88	1963.10^{b}
T4	152.05	1953.03^{b}
SEM	2.735	88.990
P-value	0.0543	0.0028

^{ab} Means in the same column with different superscripts differ significantly ($P < 0.05$). SEM = Standard error of means and g = grams. T1 is a control, T2, T3, and T4 contains 10%, 15% and 20% *Mucuna pruriens* seed meal as per DM intake, respectively.

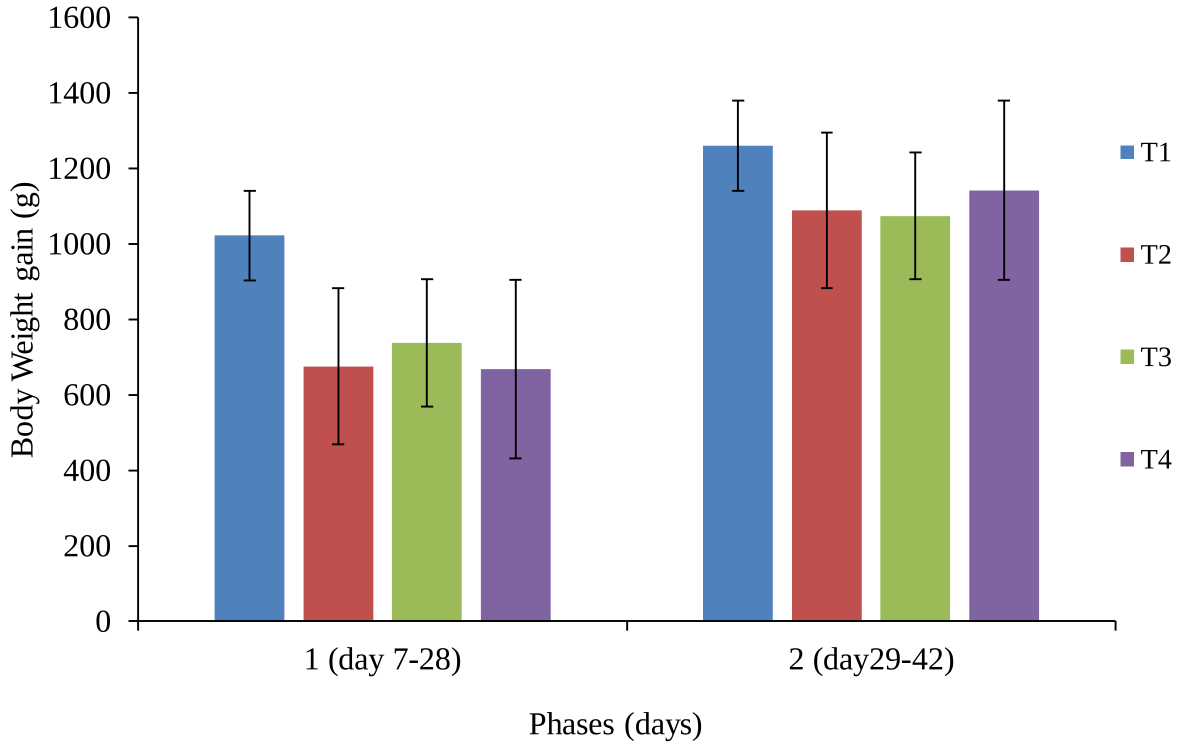

Figure 3.1: Body weight gain of broilers fed varying inclusion levels of velvet bean seed meal in starter and finisher feeding phased. T1, T2, T3, and T4 contain different levels (0, 10, 15, and 20%) velvet bean seed meal.

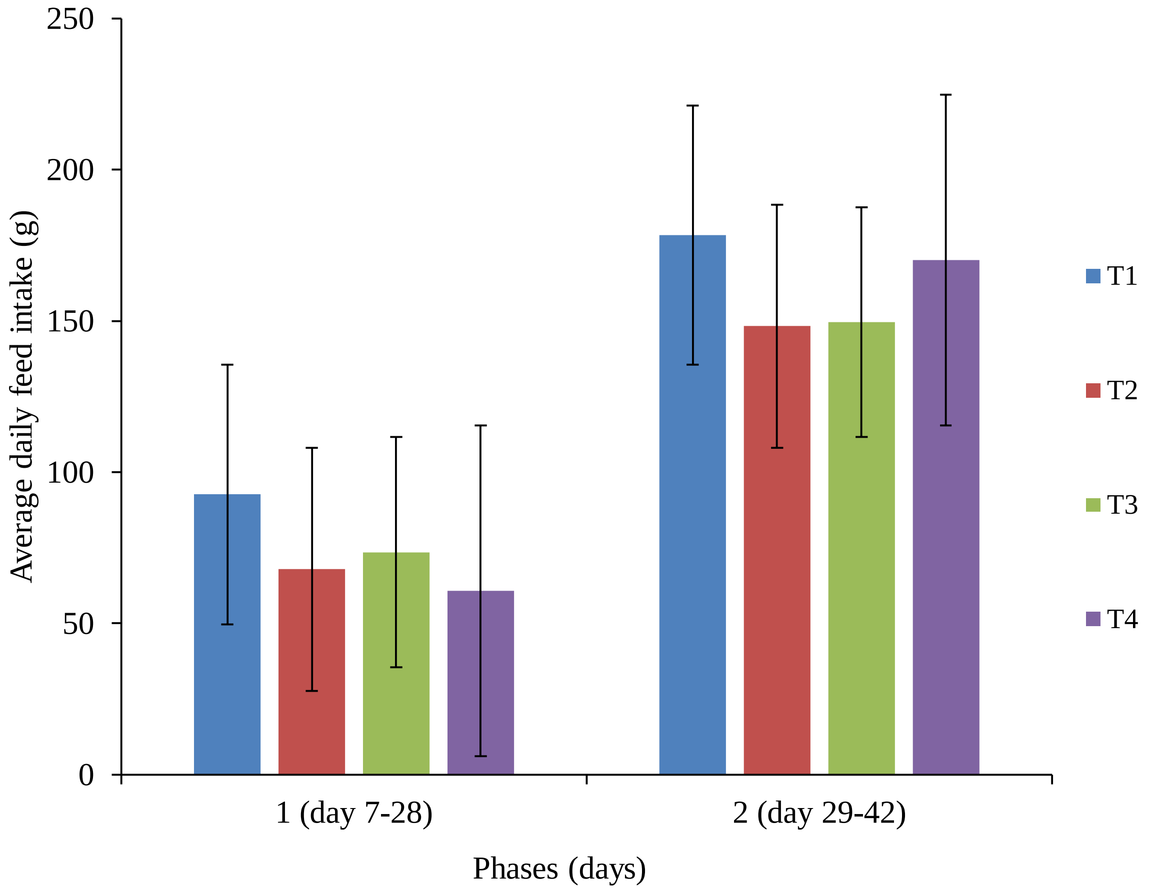

Figure 3.2: Average daily feed intake of broilers fed varying inclusion levels of velvet bean seed meal in starter and finisher feeding phases. T1, T2, T3, and T4 contains different levels (0, 10, 15, and 20%) velvet bean seed meal.

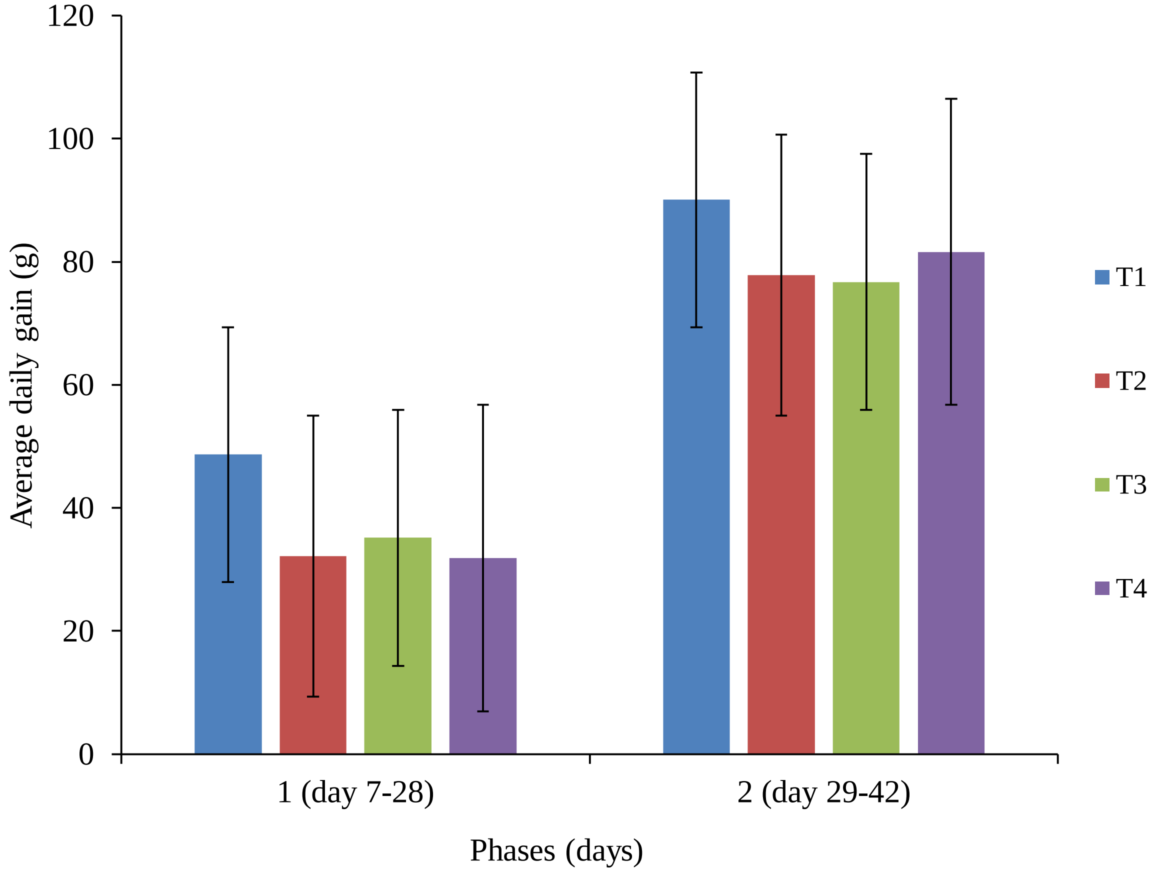

Figure 3.3: Average daily gain of broilers fed varying inclusion levels of velvet bean seed meal in starter and finisher feeding phases. T1, T2, T3, and T4 contains different levels (0, 10, 15, and 20%) velvet bean seed meal.

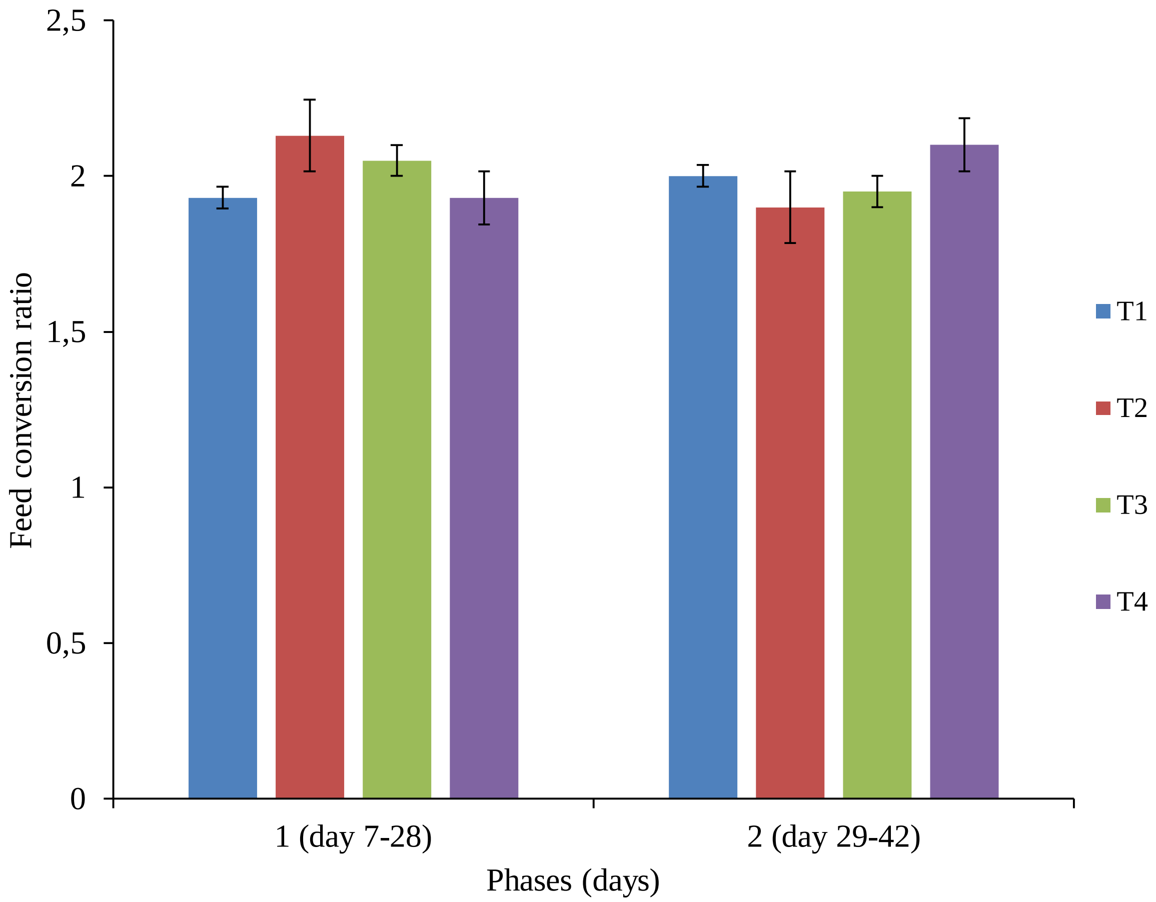

Figure 3.4: Feed conversion ratio of broilers fed varying inclusion levels of velvet bean seed meal in starter and finisher feeding phases. T1, T2, T3 and T4 contains different levels (0, 10, 15, and 20%) velvet bean seed meal.

Table 3.7: Least square mean of mortality rate from broilers in starter (Phase 1) and finisher (Phase 2) fed varying inclusion levels of velvet bean seed meal

Treatments	**Mortality (%)**	
	Phase 1: (7 to 28 days)	Phase 2: (29 to 42 days)
T1	21.45[a]	7.1
T2	0[b]	0
T3	0[b]	0
T4	0[b]	3.55
SEM	3.259	3.259

[ab] Means in the same column with different superscripts differ significantly ($P < 0.05$). SEM = Standard error of means, T1, T2, T3, and T4 contains 0, 10%, 15% and 20% *Mucuna pruriens* seed meal as per DM intake, respectively.

3.4. Discussion

Among all treatments, body weight gain was higher in broilers fed a control diet; this means that inclusion of velvet bean seed meal decreased weight gain. These findings are in line with those by Carew *et al*. (2003) where body weight gain of broilers decreased after feeding diets with velvet bean seed meal. Similarly, Del Carmen *et al*. (1999) and Jayaweera *et al*. (2007) indicate that not only raw seeds can decrease body weight gain but 20-25% inclusion of processed velvet bean seeds into a diet decreases weight gain. These findings contradict results of Emenalom and Udedibie (1998) and Del Carmen *et al*. (1999) where inclusion of 10% heated velvet bean seeds showed better growth than 20% inclusion. The negative effect of velvet bean inclusion was observed in both phases of feeding. This suggests that some anti-nutritional factors (ANFs) in velvet bean seeds might not be heat labile (Emenalo *et al*., 2005; Gurumoorthi and Uma, 2011) as heat processing results in detoxification of the ANFs, better protein absorption, better accessibility of the amino acids in the diets and greater palatability (Tuleun and Igba, 2008).

The inclusion of VBSM decreased feed intake. These finding are in line with results by Del Carmen *et al*. (1999) where feed intake was reduced at 20% and 10% inclusion levels of cooked and toasted velvet meal meals, respectively. On the other hand, Jayaweera *et al*. (2007) found that feed intake was not affected by velvet bean seed meal inclusion. In both Phases 1 and 2, broilers that were fed a control diet had high feed intake compared to those on diets with VBSM inclusions. These results agree to the findings reported by Emenalom and Udedibie (1998) and Mugendi *et al*. (2010), who concluded that the differences in feed intake may be due to the level of seed treatment and the processing techniques. In the current study, low feed intake may have been induced by unpalatability of diets due to completely incapacitated ANFs in VBS. Besides their unpalatability; tannins, phenolic, phytic acid and oxalate among other ANFs, inhibit pancreatic trypsin activity and prevent proper digestion of the dietary proteins and they also bind to the endogenous and exogenous protein, available enzymes and cations (Anhwange *et al*., 2004; Gupta *et al*., 2015; Proietti *et al*., 2015) thus, results in decreasing the intake (Anhwange *et al*., 2004).

Feed conversion ratio (FCR) is the most important parameter for broiler producers as it measures the how efficient a bird convert feed into increased body mass. In the current study the diets did not influence feed conversion ratio (FCR). These discoveries are supported by Tuleun *et al*. (2011), who observed that the FCR of broilers fed toasted and cooked VBSM up to 20% dietary inclusion level were comparable with broilers fed control diet. However, the current results contradict with observation by Jayaweera *et al*. (2007), where FCR of broilers fed increasing levels of velvet bean seed meal were higher compared to control. The poor FCR of broilers in T2 at Phase 1, and T4 at phase 2 was observed. At the starter Phase of feeding, broilers still strive to adapt to diets compared to older broilers at finisher phase feeding (Ratika *et al*., 2018). Few studies have indicated that the enhancement in FCR of broilers on diets containing heat treated VBS brings them to balance with that of maize-soybean (Emenalom and Udedibie, 1998; Jayaweera *et al*., 2007; Tuluen *et al*., 2008).

A very high mortality (28.6%) was observed in treatment 1 (control diet) through the entire experiment and also 21.45% mortality in phase 1 compared to other treatments with increasing levels of velvet bean seed meal. The current findings are not in line with results of Del Carmen *et al*. (1999), where mortality of broilers fed 10% heated VBSM did not differ from those of treatment 1. While in other studies by Emenalom and Udedibie, (2005) together with Ani (2008), no mortality was recorded in all the treatments where the inclusion of VBSM was from 0 to 20%. Every broiler producer wishes to reach high final body weight after each cycle in order to get good returns. Therefore, good quality nutrition and management should be attained. In this study, broilers in the control diet reached a higher (2438 g) final weight compared to other treatments. Jayaweera *et al*. (2007) also finds similar results whereby the final body weight decreases with incremental levels of VBSM in the diets.

3.5. Conclusion

Inclusion of VBSM in diets compromised growth rate of broilers. Diets had no effect on feed conversion ratio in all treatments including the control. However, the mortality rate was very low in broilers fed 20% velvet bean seed meal containing-diets and no mortality was seen on broilers fed diets 10 and 15% velvet bean seed meal. Due to negative effect of diets with velvet bean seed meal on growth performance, it can be concluded that it is not applicable compared to diets with soybean meal. It was suspected that processing method did not completely deactivate the ANFs, thus they lowered intake and growth rate, hence further research needs to focus on processing times and methods.

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Chapter 4: The effect of feeding varying inclusion levels of heat processed velvet bean (*Mucuna pruriens*) seed meal on physicochemical attributes of broiler meat

(*Some parts were submitted to South African Journal of Animal Science*)

Abstract

The aim of this study was to determine the effect of feeding varying inclusion levels of heat processed VBSM on pH_{24}, colour (L*-Lightness, a*-Redness, and b*-Yellowness), Warner-Bratzler shear force (WBSF), thawing loss and cooking loss of broiler meat. Recently the consumers has been conscious about the quality of chicken meat from both locally produces and exported. Furthermore, the quality of broiler meat cuts is the main factor that contributes to consumer's preferences on purchasing. Twenty birds per treatment were randomly selected and fasted for five hours with water offered *ad libitum*. Then birds were slaughtered and dressed following usual commercial procedures at Fort Cox poultry abattoir. The meat pH_{24}, colour, WBSF, TL, and CL measurements were performed on 80 breast and 80 thigh muscles of broiler birds fed diets containing control (0), 10, 15 and 20% VBSM at Meat Science Laboratory at the Department of Livestock and Pasture Science, University of Fort Hare. Data was analysed statistically using Analysis of Variance using a Generalized Linear Model (GLM) Procedure of SAS version 9.1 (2003). Mean separation was done using Least Significance Difference (LSD) test. Carcass weight from broilers fed a control diet was higher ($P < 0.05$) than other treatments. The diets with varying inclusion levels of velvet bean seed meal did not have an effect of dressing percentage. Diet comprising of 10% VBSM promoted higher ($P > 0.05$) breast pH_{24} than the breast from broilers fed T1 and T3. The diets did not have an effect ($P > 0.05$) on thigh pH_{24}. Thigh pH_{24} (6.03 – 6.15) values across all treatments were found to be higher ($P < 0.05$) than that of a breast pH_{24} (5.75 – 5.83). Breast lightness, redness, and yellowness were not ($P > 0.05$) influenced by diets. The thigh lightness and redness were also not ($P > 0.05$) influenced by diets. Diet T1 promoted higher

(12.16) thigh yellowness compared to those from diets with VBSM. Breasts shear force (14.20) was higher (P < 0.05) in broilers fed diet T4, than diet T1. Diets had no effect (P > 0.05) on thigh's shear force. Generally, the force applied on breast meat was higher (11.73-14.20N) across all treatments compered to force (7.47 - 8.42 N) applied on thigh meat. Breast meat from broilers fed diets T2 and T4 promoted higher (P < 0.05) thawing loss compared to the ones offered diet T1 and T3. The diets had no effect (P > 0.05) on thawing loss of thigh meat. Breast meat thawing loss was higher (P < 0.05) than thigh meat among different treatment diets. Cooking loss of breast meat from broilers fed diet T1 and T2 was higher (P < 0.05) than breast meat of broilers fed diets T3 and T4. Broilers fed a control diet had higher (P < 0.05) cooking loss of thigh meat compared to those fed diets T4. Between breast and thigh across all treatments, thigh meat had higher cooking loss (21.40 - 25.69) than breast meat (18.33 - 21.26). Therefore, it was concluded that the VBSM can be included in the broiler chicken diets up to the level of 15% without affecting the quality of meat. In addition, the breast meat from broilers fed velvet bean seed meal had better quality compared to thigh meat.

Keywords: Cooking loss, meat pH, muscle types, tenderness.

4.1. Introduction

Some dietary ingredients usually have an effect on the animal products such as meat yield and quality (Guerrero *et al*., 2013; Mir *et al*., 2017), and the same is expected of *Mucuna pruriens*. The addition of natural feed ingredients in broiler diets improves the quality of the diet and results in better quality of meat (Ahmed, 2017; Altmann *et al*., 2018). In addition, some studies have shown that seeds bioactive compounds such as flavonoids, tannins, condensed tannins, oxalate, and phytic acid are potent antioxidants which contribute largely to good meat quality (Seeram *et al*., 2005; Reddy *et al*., 2007). Besides the diets, there are also various factors that may affect the quality of meat such as the external environment, broiler genetics, and broiler management and pre-slaughter stress (Mir *et al*., 2017; Rindhe *et al*., 2018), and these factors should be monitored and minimised where possible.

The growing demand by health conscious consumers for broiler meat of good quality (Al-Hassan *et al*., 2014) has resulted in pressure on nutritionists to explore other potential indigenous seeds such as VBS that can be used as an alternative to currently available expensive conventional protein sources. These alternative protein sources are envisaged to provide adequate levels of crude protein to support optimal growth rate, feed efficiency and meat quality (Petracci and Cavani, 2012). These protein sources should be able to be produced at a low cost by resource poor emerging or smallholder famers. With the threat of climate change likely to severely impact on poorly resourced farmers, drought tolerant legumes such as velvet bean would be ideal as a protein source in chicken feeds.

For an alternative protein source to have a significant impact, and become a possible substitute ingredient in diet formulation its effect has to transition from feed intake, growth through to meat quality. Physicochemical attributes of meat are the most important in determining the quality of broiler meat. According to Petracci and Fletcher (2002), meat should have a desirable colour that is uniform throughout the entire cut and this can mainly be influenced by the pH of the meat. Meat colour is related to the levels of the protein pigments (myoglobin and haemoglobin) present in the muscle which are affected by anti and post mortem conditions and type of storage (Mancini and Hunt, 2005). For normal broiler meat, it is recommended that the lightness should range between 50 to 56, where a value less than 50 corresponds with darker meat, while that greater than 56 results in pale meat (Petracci *et al*., 2004). Anti-nutritional factors in diets have an effect on meat colour since they are a precursor for the synthesis of haemoglobin pigments (Priolo and Vasta, 2007; Moyo *et al*., 2014).

The histological, bio-physical and biochemical characteristics of muscle fibres play a key role on meat quality (Picard *et al*., 2006). The breast and thigh meat comprise of different muscle fibres, sizes, and density, which contribute mainly to tenderness of the meat. It is also believed that WBSF has been linked to the location of the muscle (FAO, 2018). Therefore, there is a need to investigate how diets comprising of VBSM affect the quality of meat muscle types (breast and thigh), since it is believed that the size and number of muscle fibre in a muscle are factors that influence meat quality (Tůmová and Teimouri, 2009).

Mucuna pruriens seed meal as a protein source in broiler feed in Asia and East Africa has been widely investigated (Del Carmen *et al*., 1999; Iyayi and Taiwo, 2003; Nyirenda *et al*., 2003; Jayaweera *et al*., 2007; Ani, 2008; Vadivel and Pugalenthi, 2010; Mangwiro *et al*., 2013). But the focus was on its potential benefits as an alternative source of protein on the growth of birds, little has been reported on its effect on the quality of meat. To our information, only one study reported the effect of feeding *Mucuna pruriens* seed meal on Guinea Fowl meat quality attributes (Dahouda *et al*., 2009), thus there is a dearth of knowledge on the effect *Mucuna pruriens* seed meal on broiler meat quality. There is need to investigate the effect of *Mucuna pruriens* seed meal on physicochemical attributes of chicken meat. It was hypothesised that feeding varying inclusion levels of heat processed VBSM seed meal does not alter physicochemical attributes of broiler meat. Therefore, the objective of this study was to determine the effect of feeding varying inclusion levels of heat processed VBSM on physicochemical attributes of broiler breast and thigh meat.

4.2. Materials and Methods

4.2.1. Study site description

The analysis was conducted at the Meat Science Laboratory of the department of Livestock and Pasture science at the University of Fort Hare in Alice. Alice is situated in Raymond Mhlaba Local Municipality under Amatole District in the Eastern Cape province of South Africa. Ethical consideration is as explained in the previous Section 3.2.1 of Chapter 3.

4.2.2. Feed preparation and formulation

Feed preparation and formulation is as explained in the previous Section 3.2.3 of Chapter 3.

4.2.3. Experimental design, broiler feeding and management

Experimental design, broiler feeding and management are as described in the previous Section 3.2.4 of Chapter 3.

4.2.4. Broiler vaccination and slaughter procedure

Broiler vaccination and slaughter procedure is as explained in the previous Section 3.2.5 and 3.2.6 of Chapter 3, respectively.

4.2.5. Meat pH and colour measurements

The meat pH measurement was performed 24 h after slaughter on 80 breast and 80 thigh muscles of broiler chickens using a pH meter (Crison pH 25, Crison instruments, S.A., Alella, Spain). The pH meter was calibrated with pH 4 and pH 7 standard solutions. Colour of the meat (L* = Lightness, a* = Redness and b* = Yellowness) was also determined 24 h after slaughter using a Minolta colour-guide 45/0 BYK-Gardener GmbH machine, with a 20 mm diameter measurement area and illuminant D65-day light, 10º observation angle. Colour components were determined during blooming after removal of the fillets from the packaging, and exposure to air for 30 min to allow the oxygenation of myoglobin as described by Tapp *et al*. (2011). Three readings were taken by rotating the Colour Guide 90º between each measurement, in order to obtain a representative average value of the colour.

4.2.6. Thawing loss and cooking loss determination

The 80 breast and 80 thigh meat samples that were used for thawing and cooking loss determination were frozen at -20°C for seven days. A day before preparation, all samples were weighed using digital scale and thawed over 24 h at 4°C and weighed again after thawing as described by Sekali *et al*. (2016). The breast and thigh meat samples were vacuum packed in plastic bags and cooked using a water bath at 85ºC for 45 min (Ding *et al*., 2010). Raw and cooked weights were recorded. Percentage thawing loss and cooking loss was calculated as described by Honikel (1998) as follows:

$$\text{Thawing loss} = \frac{\text{Weight before thaw} - \text{Weight after thaw}}{\text{Weight before thaw}} \times 100\%$$

$$\text{Cooking loss} = \frac{\text{Weight after thawing} - \text{Weight after cooking}}{\text{Weight after thawing}} \times 100\%$$

4.2.7. Meat Shear force value determination

The tenderness of 80 breast and 80 thigh meat samples was determined using Instron-Warner-Bratzler Shear Force (WBSF) device. Following cooking, four sub-samples of 10 mm core diameter were cored parallel to the grain of the meat. The samples were sheared perpendicular to the fibre direction using a Warner-Bratzler shear device mounted on an Instron, model 3344, Universal Testing. The mean maximum load in Newton's (N) was recorded for the batch.

4.2.8. Statistical analysis

The effect of varying inclusion levels of velvet bean seed meal on carcass weight, dressing percentage, broiler breast and thigh meat pH, L*, a*, b*, thawing loss, cooking loss and shear force value were statistically analysed using the procedure of SAS (2006). The differences among means were tested for significance ($P<0.05$) using Least Significance Difference (LSD) test option of SAS (2006). The statistical model used was as follows:

$$Y_{ij} = \mu + \alpha_i + \varepsilon_{ij}$$

Where Y_{ij} = response variables (carcass weight, dressing percentage, pH_{24}, L*, a*, b*, WBSF, TL, CL),

μ = overall mean,

α_i = effect of dietary treatments (T1, T2, T3, and T4),

ε_{ij} = random error.

4.3. Results

4.3.1. Carcass weight and dressing percentage

Carcass weight was higher ($P < 0.05$) from broilers fed a control diet than those fed diets having 10, 15 and 20% VBSM (Table 4.1). The diets with varying inclusion levels of velvet bean seed meal did not ($P > 0.05$) have an effect on dressing percentage (Table 4.1).

Table 4.1: Least square mean of carcass weight and dressing percentage of broilers fed varying inclusion levels of velvet bean seed meal

Treatments	**Parameters**	
	Carcass Weight (g)	Dressing %
T1	1789.55^{a}	73.85
T2	1488.15^{b}	77.98
T3	1416.83^{b}	72.65
T4	1452.28^{b}	74.40
SEM	38.599	3.259
P-value	<0.0001	0.6932

^{ab} Means in the same column with different superscripts differ significantly ($P < 0.05$). SEM = Standard error of means and g = grams. T1 is a control, T2, T3, and T4 contains 10%, 15% and 20% *Mucuna pruriens* seed meal as per DM intake, respectively.

4.3.2. Meat pH_{24}

Breast from T2 had higher (*P* < 0.05) meat pH_{24} than those from T1 and T3 (Table 4.2). The diets did not influence thigh meat pH_{24} (Table 4.3). Thigh meat pH_{24} (6.03 – 6.15) values across all treatments were found to be higher than that of a breast meat pH_{24} (5.75 – 5.83) (Table 4.4).

4.3.3. Meat colour

Breast meat colour (Lightness, redness and yellowness) were not (*P* > 0.05) influenced by diets (Table 4.2). The thigh meat lightness and redness were also not (*P* > 0.05) influenced by diets. The control diet resulted higher (*P* < 0.05) thigh meat yellowness compared to those from diets with velvet bean seed meal (Table 4.3). Breast meat colour differed significantly (*P* < 0.05) from thigh meat colour across treatments (Table 4.4).

4.3.4. Meat shear force value

Breast's Warner-Bratzler shear force was higher (*P* < 0.05) in broilers that were fed a diet T4 than the control diet (Table 4.2). Diets had no effect (*P* > 0.05) on thigh meat shear force values (Table 4.3). Generally, the force applied on breast meat was higher (11.73-14.20 N) across all treatments compared to force (7.47 - 8.42N) applied on thigh meat (Table 4.4).

4.3.5. Meat thawing loss

Breast meat from broilers fed diets T2 and T4 had a significantly higher (*P* < 0.05) thawing loss than those fed diet T1 and T3 (Table 4.2). The diets had no effect (*P* > 0.05) on thawing loss of thigh meat (Table 4.3). In comparison, breast meat thawing loss was significantly higher (*P* < 0.05) than those of thigh meat among different treatment diets (Table 4.4).

4.3.6. Meat cooking loss

Cooking loss of breast meat from broilers fed diet T1 and T2 was found to be higher (*P* < 0.05) than T3 and T4 (Table 4.2). Broilers fed control diet resulted in higher (*P* < 0.05) cooking loss of thigh meat compared to those fed diet T4 (Table 4.3). Across all treatments, thigh meat had higher cooking loss (21.40 - 25.69) than breast meat (18.33 - 21.26) (Table 4.4).

Table 4.2: Least square mean (±standard error) of physicochemical attributes of breast meat from broilers fed varying inclusion levels of velvet bean seed meal

Treatment	**Number of samples**	**Physicochemical Attributes**						
		pH_{24}	L*	a*	b*	WBSF	TL (%)	CL (%)
T1	20	5.75^{b}±0.071	47.45±3.754	5.90±1.674	11.29±2.711	11.73^{b}±2.058	7.46^{b}±1.605	21.26^{a}±1.858
T2	20	5.83^{a}±0.078	46.13±3.332	6.20±1.795	10.86±1.376	12.52^{ab}±2.552	8.82^{a}b±1.908	20.22^{a}±1.416
T3	20	5.76^{b}±0.064	46.13±2.899	7.12±2.251	11.43±1.724	13.04^{ab}±2.254	8.01^{b}±2.139	18.72^{b}±2.417
T4	20	5.79^{ab}±0.075	45.75±2.376	6.17±1.141	11.71±1.946	14.20^{a}±3.740	9.66^{a}±1.521	18.33^{b}±1.307
P-value		0.0062	0.3415	0.1495	0.5976	0.0405	0.0016	<0.0001

^{ab} Means in the same column with different superscripts differ significantly (P <0.05). L* = Lightness, a* = Redness, b* = Yellowness, WBSF = Warner-Bratzler Shear Force, TL(%) = Percentage Thawing loss, CL(%) = Percentage Cooking loss. T1 is a control, T2, T3, and T4 contains 10%, 15% and 20% *Mucuna pruriens* seed meal as per DM intake, respectively.

Table 4.3: Least square mean (±standard error) of physicochemical attributes of thigh meat from broilers fed varying inclusion levels of velvet bean seed meal

Treatment	Number of samples	Physicochemical Attributes						
		pH_{24}	L*	a*	b*	WBSF	TL (%)	CL (%)
T1	20	6.03±0.142	48.37±2.844	6.48±2.056	12.16^{a}±1.641	8.42±1.614	5.64±2.552	25.69^{a}±2.938
T2	20	6.11±0.157	48.42±2.701	6.14±1.445	10.25^{b}±1.597	7.90±1.647	6.30±2.572	23.06^{ab}±3.100
T3	20	6.10±0.238	47.41±2.519	6.71±1.516	10.51^{ab}±2.246	8.28±1.489	6.30±1.929	23.92^{ab}±1.936
T4	20	6.15±0.150	47.46±3.734	6.54±2.353	9.35^{b}±3.114	7.47±0.872	6.26±1.974	21.40^{b}±7.694
P-value		0.2019	0.5559	0.8038	0.0018	0.1629	0.7517	0.0296

^{ab} Means in the same column with different superscripts differ significantly ($P < 0.05$). L* = Lightness, a* = Redness, b* = Yellowness, WBSF = Warner-Bratzler Shear Force, TL(%) = Percentage Thawing loss, CL(%) = Percentage Cooking loss. T1 is a control, T2, T3, and T4 contains 10%, 15% and 20% *Mucuna pruriens* seed meal as per DM intake respectively.

Table 4.4: Least square mean of physicochemical attributes of breast and thigh meat from broilers fed varying inclusion levels of velvet bean seed meal

Treatment	**Muscle type**	**Physicochemical attributes**						
		pH_{24}	L*	a*	b*	WBSF	TL	CL
T1	Breast	5.75^{c}	47.45^{ab}	5.90^{b}	11.29^{abc}	11.73^{b}	7.46^{cd}	21.26^{bcd}
	Thigh	6.03^{b}	48.37^{a}	6.48^{ab}	12.16^{a}	8.42^{c}	5.64^{e}	25.69^{a}
T2	Breast	5.82^{c}	46.13^{b}	6.20^{ab}	10.86^{abc}	12.52^{b}	8.82^{ab}	20.22^{cde}
	Thigh	6.11^{a}	48.42^{a}	6.14^{ab}	10.25^{cd}	7.90^{c}	6.30^{de}	23.06^{b}
T3	Breast	5.75^{c}	46.13^{b}	7.12^{a}	11.43^{abc}	13.04^{ab}	8.01^{bc}	18.72^{de}
	Thigh	6.10^{ab}	47.41^{ab}	6.71^{ab}	10.51^{bcd}	8.28^{c}	6.30^{de}	23.92^{f}
T4	Breast	5.78^{c}	45.75^{b}	6.17^{ab}	11.71^{ab}	14.20^{a}	9.66^{a}	18.33^{e}
	Thigh	6.15^{a}	47.46^{ab}	6.54^{ab}	9.35^{d}	7.47^{c}	6.26^{de}	21.40^{bc}
SEM		0.030	0.684	0.407	0.474	0.488	0.060	0.767

^{abcdef} Means in the same column with different superscripts differ significantly ($P < 0.05$). pH_{24} = pH after 24 hours, L* = Lightness, a* = Redness, b* = Yellowness, WBSF = Warner-Bratzler Shear Force, TL(%) = Percentage Thawing loss, CL(%) = Percentage Cooking loss. T1, T2, T3, and T4 contains 0, 10, 15 and 20% inclusion levels of *Mucuna pruriens* seed meal, respectively.

4.4. Discussion

High carcass weight was observed on broilers fed a control diet than those fed diets having 10, 15 and 20%VBSM. Similar trend was also observed by Emenalom and Udedibie, (2005); Iyayi *et al*. (2006). The smaller carcass weight in broilers fed diets with VBSM could have possibly resulted from their smaller final weight. This trend is supported by Tuleun & Igba, (2008), where it was stated that the carcass weight is determined by the visceral produced. The dressing percentages were not influenced by diets. Diet comprising of 10% VBSM had higher breast meat pH_{24} than the breast from broilers fed diets having 0 and 15% VBSM, however the pH_{24} values of breast meat in all treatments were in the normal range of 5.75 to 5.83. This is supported by Hambrecht *et al*. (2004), whereby generally over 24 hours after slaughter, the pH in the chicken meat decreases from approximately 7.0-7.2 down to a range of 5.5-5.8 for normal meat. In the study by Dahouda *et al*. (2009) it was also observed that the breast meat pH_{24} of Guinea Fowls fed raw, cooked, and toasted velvet bean seed meal varied from 5.7-5.8. In the current study, high thigh meat pH_{24} (6.03-6.15) values across all treatments were observed compared to breast meat pH_{24} (5.75-5.83). This is supported by FAO (2018), whereby it was observed that the location of the muscle somehow has an effect on the quality of meat. And also the broiler nutrition has a significant impact on broiler meat quality (Guerrero *et al.* 2013; Mir *et al*. 2017). This unacceptable thigh meat pH_{24} values may be the result of the production of lactic acid from poor breakdown of glycogen after slaughter compared to breast meat (Lammers *et al*., 2007; Barbut *et al*., 2008), since meat pH is widely associated with muscle type and sizes of muscle fibres (Choi *et al*., 2016). Choi and Kim (2009) noted that the smaller muscle fibres are highly associated with the pale, soft and exudative (PSE) condition, which is affected mainly by pH.

Increasing levels of VBSM inclusions did not show any influence on lightness, redness and yellowness of breast meat. The thigh meat lightness and redness were also not influenced by the diets. A similar trend was found in other related legume seeds, whereby Laudadio and Tufarelli (2010), observed that the inclusion of peas did not have an effect on breast and drumstick colour of broiler meat. The control diet resulted in higher thigh meat yellowness compared to those from diets with VBSM. However, these findings contrast with Laudadio *et al*. (2011), who reported that thigh meat from broilers fed faba bean diets had higher yellowness when compared to broilers fed the soybean meal diet which was the control. This difference could be attributed to the influence of anti-nutritional factors (ANF) and processing method found in VBS, which in the current study the antioxidant properties may not have influenced the yellowness value (Priolo and Vasta 2007; Moyo *et al*., 2014). Since antioxidants ultimately alters muscle colour, possibly by reducing haemoglobin oxidation that causes pigment supply in body tissues (Reddy *et al*., 2007). In addition, the findings by Laudadio *et al*. (2011), on thigh meat yellowness may also have been attributed to the fact that yellow maize was not part of the ingredients used during formulation unlike in the current study. The higher yellowness in thighs from broilers fed a control diet in the current study may be due to a high concentration of xanthophyll pigment in the diet since the diets contained yellow maize and soymeal as basal ingredients. Perez-Vendrell *et al*. (2001) indicated that the colour of poultry meat is influenced by xanthophyll pigment present in the diet of birds that is deposited in the subcutaneous fat and skin.

The inclusion of VBSM did not affect the tenderness of thigh meat. In the current study it was found that the breast meat was tougher than thigh meat as expected. This may possibly be due to the difference in amount and quality of connective tissue fibres in both breast and thigh muscle, since it is believed that the size and number of muscle fibre in a muscle are factors that influence meat tenderness (Tůmová and Teimouri, 2009). Tenderness (WBSF) of breast meat increased with increasing inclusion levels of VBSM, where in T4, higher force (14.20 N) was applied to cut breast meat while in T1 lesser force (11.73 N) was applied.

These results may have been stimulated by the presence of anti-nutrients that are heat liable in VBS, which triggers oxidation in meat (Seeram *et al*., 2005; Reddy *et al*., 2007), since tough meat is due to oxidation of myofibrillar protein, which promote combination of muscle fibres (Harris *et al*., 2001; Morán *et al*., 2012). Currently there is no traceable evidence on the analysis of broiler meat fed VBSM, however, the determination of tenderness through sensory evaluation was done in few studies. In these studies it was observed that the VBSM in diets seemed not to have an effect on tenderness, juiciness and flavour in the broiler breast (Adzitey *et al*., 2010). While on other related legume seeds that were fed to broilers, Milczarek *et al*. (2016), observed that the inclusion of faba beans to a broiler diet had an effect on texture properties of which the meat was found to be tenderer. It is suggested that the softness on chewing breast meat is subjective to factors such as sarcomere shortening during rigor mortis, the amount and solubility of connective tissues, the rate of post mortem energy metabolism, and post mortem proteolysis of myofibrillar proteins (Warner *et al*., 2010). What lead to more strength in muscle is the muscle fibres where they eventually decrease tenderness of the meat, because many myofibrils are arranged in register across the fibres (Thu, 2006).

Breast meat from broilers fed diets containing 10 or 20% VBSM had higher thawing loss compared to the ones offered diets containing 0 or 15% VBSM. However, in the study by Dahouda *et al*. (2009), the breast meat thawing loss on Guinea Fowls fed a diet containing 20% VBSM was found to be lower. Since Guinea Fowls might be comparable to indigenous chickens, therefore the ability of their muscle fibres to retain intrinsic water becomes poor because of age at slaughter (Listrat *et al*. 2016; Soglia *et al*. 2016). Breast meat thawing loss was higher than those of thigh meat among the different diets. Generally, since thawing loss is associated with size of muscle fibres, of which they may affect the ability of meat to bind water. Most water in body cells is held in myofibrils and that water is reserved by capillary forces that are generated by the arrangement of thin and thick fibres within the myofibril (Huff-Lonergan and Lonergan, 2005; Soglia, 2016).

Breast meat cooking loss from broilers fed diet having 0 and 10% VBSM was found to be higher than those from broilers fed diets containing 15 and 20% VBSM. This suggests that increasing the levels of 15 to 20% VBSM in a diet lowers the amount of substance lost during cooking. These results correspond with the findings by Dahouda *et al*. (2009), where lower cooking loss of breast meat from Guinea Fowls fed 20% cooked, and toasted velvet bean seed meal was observed. Furthermore, the drippings from cooked meat include nitrogenous and non-nitrogenous extractives, fats, salts, and water which are beneficial to meat consumers (Adzitey *et al*., 2010). In comparison between both muscle types across all treatments, thigh meat had higher cooking loss than breast meat. This may suggest that the repulsion of structures within thigh meat myofibrils is diminished, allowing the shrinkage of the meat (Strydom *et al*., 2016). Generally, since it is known that thigh meat contains higher fat than breast meat, therefore meat containing a high percentage of fat cooked gives greater cooking losses than higher muscular meat (Rammouz *et al*., 2004).

4.5. Conclusion

The current study showed that the dressing percentage is not affected by diets, although inclusion of VBSM decreases carcass weight. Most of the physicochemical attributes were not affected by diets, this includes thigh pH_{24}, lightness, redness, thawing loss and breast colour. However, toughness of breast from broilers fed 20% velvet bean seed meal was higher than the control diet. Breasts had higher thawing loss than thigh among different treatment diets. Therefore, it can be concluded that the VBSM can be included in the broiler diets up to the level of 15% without affecting most meat quality parameters.

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Chapter 5: General discussion, conclusion and recommendation

5.1. General discussion

The broad objective of the current study was to determine the effect of feeding varying inclusion levels of heat processed VBSM on growth performance and physicochemical attributes of cobb-500 broiler chickens. The null hypothesis tested was that feeding varying inclusion levels of heat processed VBSM does not affect broilers growth performance and physicochemical attributes of broiler meat. Before improving growth rate, feed conversion efficiency and meat quality by indigenous legume seeds such as VBS, the challenges of using velvet bean seeds should be fully understood.

The hypothesis tested in Chapter 3 was that feeding varying inclusion levels of heat processed VBSM does not affect broilers feed intake and growth performance. However its inclusion decreased feed intake and body weight gain. These results were in line with the finding of (Emenalom and Udedibie, 1998; Del Carmen *et al*. 1999; Carew *et al*. 2003; Jayaweera *et al*. (2007); Mugendi *et al*. 2010) where it was observed that inclusion of 10-25% inclusion of processed VBS into a diet decreases weight gain. This suggests that some ANFs in VBS might not be heat labile (Emenalo *et al*., 2005; Gurumoorthi and Uma, 2011). The low intake may have been induced by unpalatability of diets due to completely denatured ANFs in VBS. Besides being unpalatable, ANFs inhibit pancreatic trypsin activity and prevent proper digestion of the dietary proteins (Anhwange *et al*., 2004; Gupta *et al*., 2015; Proietti *et al*., 2015). A very high mortality (28.6%) was observed in treatment 1 through the entire experiment. The current findings are not in line with results by Del Carmen *et al*. (1999), Emenalom and Udedibie (2005); Ani (2008), where less than 10% mortality was seen.

In Chapter 4 it was hypothesised that the feeding varying inclusion levels of heat processed VBSM does not affect physicochemical attributes of broiler meat. The hypothesis tested was true on thigh meat $pH_{24,}$ lightness, redess, WBSF and cooking loss. The study on the effect of velvet bean seed meal on Guinea Fowls meat quality was carried by Dahouda *et al*. (2009), and similar trend was observed on meat pH while contradicts with colour, and cooking loss. However breast meat was in acceptance range of 5.5 to 5.8 meat pH. Choi and Kim (2009) noted that the smaller muscle fibres are highly associated with the pale, soft, and exudative (PSE) condition which is affected mainly by pH. Cooking loss of breast meat from broilers on T1 and T2 was higher than those from other treatments. This may suggest that the repulsion of structures within thigh meat myofibrils is diminished, allowing the shrinkage of the meat (Strydom *et al*., 2016).

5.2. General conclusion

The varying levels of VBSM in the diets decreased feed intake, body weight gain and the final body weight of broilers. However the diets did not have an effect on feed conversion ratio in all treatments. No mortality rate was seen on broilers fed diet T2 and T3. The breast meat colour, thigh meat pH_{24} and dressing percentage were not affected by diets, although inclusion of velvet bean seed meal decreases carcass weight. The thigh meat lightness and redness were also not influenced by diets. Breast meat was tougher in broilers that were fed diet T4. Velvet bean seed meal can be included in the broiler diets up to the level of 15% without affecting the quality of meat.

5.3. Recommendations

- Oven heat processing method did not completely deactivate the ANFs, thus they lowered intake and growth rate, hence further research needs to focus on processing times and methods.

- For further research on the immune response of broilers fed VBSM diet is necessary due to very low mortality rate observed.
- *Mucuna pruriens* seeds are known to decreases cholesterol levels in animal's body. However this claim is not clear, but the investigation of its effect of fatty acid profiles of meat may bring solid arguments.

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6.0. Appendices

Appendix 6.1: Ethical clearance certificate

University of Fort Hare
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ETHICAL CLEARANCE CERTIFICATE
REC-270710-028-RA Level 01

Certificate Reference Number:	GAJ041AMTH01
Project title:	**Effect of feeding Velvet bean (Mucuna pruriens) seed meal inclusions on growth performance, meat quality and fatty acids profile of broiler chickens.**
Nature of Project	Masters in Livestock and Pasture
Principal Researcher:	Makiwa Simeon Mthana
Supervisor:	Mr C Gajana
Co-supervisor:	N/A

On behalf of the University of Fort Hare's Research Ethics Committee (UREC) I hereby give ethical approval in respect of the undertakings contained in the above-mentioned project and research instrument(s). Should any other instruments be used, these require separate authorization. The Researcher may therefore commence with the research as from the date of this certificate, using the reference number indicated above.

Please note that the UREC must be informed immediately of

- Any material change in the conditions or undertakings mentioned in the document;
- Any material breaches of ethical undertakings or events that impact upon the ethical conduct of the research.

Appendix 6.2: English editing certificate

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ERTIFICATE OF EDITING

This document certifies that a copy of the dissertation whose title appears below was edited for proper English language usage, grammar, punctuation, spelling, and overall style by Dr Sindiso Zhou whose academic qualifications appear in the footer of this document. The research content and the author's intentions were not altered during the editing process.

TITLE: **THE EFFECT OF FEEDING VARYING INCLUSION LEVELS OF VELVET BEAN (*MUCUNA PRURIENS*) SEED MEAL ON BROILER GROWTH PERFORMANCE AND PHYSICOCHEMICAL ATTRIBUTES OF CHICKEN MEAT**

AUTHOR: **MTHANA, MAKIWA SIMEON** (201206944)

DATE: **7 November 2018**

EDITOR'S COMMENT

The author was advised to effect suggested corrections concerning structure, clarity of terms and expression.

Signature

PhD Applied Linguistics (UFH), MA Applied Linguistics (MSU), BA (Honours) English and Communication (MSU), DipEd English (UZ)
Professional Membership: A member of the Professional Editors Guild